

# Safety and preliminary efficacy of allogeneic bone marrow-derived multipotent mesenchymal stromal cells for Systemic Sclerosis: a phase I-II proof-of-concept clinical study

## SUPPLEMENTARY MATERIAL AND METHODS

### Systemic Sclerosis (SSc) patients inclusion and exclusion criteria

#### *Inclusion criteria*

1. Age >18 and < 70 years
2. SSc fulfilling the 2013 criteria of the American College of Rheumatology and/or European League Against Rheumatism
3. Severe and serious SSc with poor prognosis involving life-threatening with severe visceral impairment and contraindication or resistant to conventional immunosuppressive therapy according to the European recommendations of European Scleroderma Trials and Research group (EUSTAR) and European Society for Blood and Marrow Transplantation (EBMT), which are based on high doses of cyclophosphamide administered IV (either by monthly bolus for at least 6 months or by intensification and autologous haematopoietic Stem Cell transplantation) or SSc with life-threatening fibrosing pulmonary involvement, when the possibility of a lung transplant has been ruled out, with at least 6 months follow-up after completion of prior high doses of iv cyclophosphamide when they were made
4. Modified Rodnan Skin Score  $\geq 15$
5. Presence of one or more major internal organ involvement:
  - Respiratory: i) interstitial lung disease on chest X-Ray or High-resolution computed tomography (HRCT) including bronchiolar involvement, ground glass, or fibrosis and ii) Diffusion capacity of carbon monoxide (DLCO) <60% or forced vital capacity (FVC)  $\leq 70\%$  of the theoretical value, or alteration of FVC and/or total lung capacity (TLC)  $\geq 10\%$  and/or alteration of DLCO  $\geq 15\%$  within the  $12 \pm 6$  months prior to inclusion
  - Cardiac: any of the following criteria: reversible congestive heart failure, ventricular or atrial rhythm disturbances (*ie* recurrent atrial fibrillation or flutter, recurrent atrial paroxysmal tachycardia or ventricular tachycardia), second or third degree atrioventricular block, pericardial effusion
  - Renal: any of the following criteria: hypertension (two successive blood pressure readings of either systolic  $\geq 160$  mm Hg or diastolic  $> 110$  mm Hg, at least 12 hours apart), persistent urinalysis abnormalities (proteinuria, hematuria, casts), microangiopathic hemolytic anemia, new renal insufficiency (serum creatinine  $>$  upper limit of normal)

#### *Exclusion criteria*

1. Pregnancy or lack of contraception during the whole study
2. One or more severe comorbidities:
  - Respiratory: pulmonary hypertension (systolic pulmonary artery pressure (PAP)  $> 40$ mmHg on cardiac echography or mean PAP  $> 25$ mmHg on right catheterism) and/or DLCO  $< 30\%$  and/or respiratory failure (PaO<sub>2</sub>  $< 8$  kPa ( $< 60$ mmHg) and/or PaCO<sub>2</sub>  $> 67$  kPa ( $> 50$ mmHg) without oxygen)
  - Cardiac: clinical evidence of refractory congestive heart failure, left ventricular ejection fraction  $< 35\%$  (echography or magnetic resonance imaging), uncontrolled ventricular arrhythmia, or pericardial effusion with hemodynamic consequences as evaluated by an experienced echocardiographer
  - Renal: creatinine clearance  $< 40$  ml/min (measured or estimated) or cyclophosphamide-induced sequelae cystopathy
  - Liver failure as defined by a sustained 3-fold increase in serum transaminase or bilirubin
  - Uncontrolled psychiatric trouble, including substance consumption or alcohol excess
3. Active neoplasia or concomitant myelodysplasia, antecedent of neoplasia
4. Bone marrow failure: neutrophil  $< 0.5 \times 10^9/L$ , platelet  $< 50 \times 10^9/L$ , hemoglobin  $< 8$  g/dL, lymphocyte T CD4<sup>pos</sup>  $< 200 \times 10^6/L$
5. Acute or uncontrolled chronic infections, active chronic B or C hepatitis, HIV1/2 or HTLV1/2 positive serology
6. Significant exposure to bleomycin, toxic oils, vinyl chloride, trichloroethylene or silica, eosinophilia-myalgia syndrome, eosinophilia fasciitis,
7. Risk of non-compliance
8. No social security

### Intra-familial (sibling or related) allogeneic bone marrow donors

#### *Inclusion criteria*

1. Age  $\geq 18$  and  $< 65$  years
2. Intrafamilial (including spouse) relative
3. Effective contraception for women of childbearing age.

#### *Exclusion criteria*

1. Xylocaine allergy

2. Usual contraindication for bone marrow donation including no significant medical history, negative serological testing for syphilis, HIV 1 and 2, HBs, HCV, HTLV 1 and 2, and negative HIV, HBV and HCV Polymerase Chain Reaction (PCR)
3. Pregnancy or lack of contraception
4. No social security

## Summary table of procedures and examinations performed in diffused SSc patients participating in this research.

In bold: specific research procedures

In non-bolded letters: procedures related to the usual follow-up of the disease

<b>Patients = recipients</b>	<b>Eligibility assessment<sup>1</sup></b>	<b>Inclusion<sup>2</sup></b>	<b>D0= administration of MSC</b>	<b>D10</b>	<b>M1</b>	<b>M3</b>	<b>M6</b>	<b>M9</b>	<b>M12</b>	<b>M15</b>	<b>M18</b>	<b>M21</b>	<b>M24</b>
<b>Patient information/Signature of the consent form</b>	<b>X</b>												
Medical history and Autoimmune disease history;	X												
<b>Identification of intrafamilial donor</b>	<b>X</b>												
Detailed physical examination: Weight in kg; height; concomitant treatments	X		X	X	X	X	X	X	X	X	X	X	X
<b>Rodnan score</b>	<b>X</b>		<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
Laboratory tests: haematology and biochemistry;	X		X		X	X	X	X	X	X	X	X	X
<b>Viral serologies</b>	<b>X</b>		<b>X</b>			<b>X</b>			<b>X</b>				<b>X</b>
Standard immunology	X												
24-hour proteinuria and urine cytobacteriology	X		X			X	X	X	X	X	X	X	X
Gynaecological consultation Stomatology consultation	X								X				X
<b>beta-HCG</b>	<b>X</b>								<b>X</b>				<b>X</b>
<b>Chest X-ray and Electrocardiogram</b>	<b>X</b>		<b>X</b>				<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
Chest CT scan	X						X		X				X
Gating and/or echocardiography and/or myocardial MRI (if at time of echocardiography)	X						X		X		X		X
Echocardiography	X						X		X		X		X
Blood gas	X						X		X				X
PFT + DLCO	X					X	X		X		X		X
SHAQ, VAS, SF-36 score	X		X		X	X	X	X	X	X	X	X	X
ECOG performance status	X		X		X	X	X	X	X	X	X	X	X
<b>Skin biopsy (optional)</b>	<b>X</b>				<b>X</b>	<b>X</b>			<b>(X)</b>				<b>(X)</b>
Bronchoalveolar lavage and bronchial biopsy if needed	(X)												
HLA typing (if not performed previously)	X												
Daily dose of corticosteroids			X				X	X	X	X	X	X	X
<b>Validation of eligibility</b>		X											
<b>Immunological assessment</b>			<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>		<b>X</b>		<b>X</b>
<b>Lymphocyte immunophenotyping</b>			<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>		<b>X</b>		<b>X</b>
<b>Cell bank/DNA bank</b>			<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>		<b>X</b>		<b>X</b>
<b>Serum bank/Plasma bank</b>			<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<b>Alloimmunisation</b>													
- Mixed lymphocyte reaction			X		X	X							
- Anti-HLA Antibodies	X				X	X							
- Microchimerism			X		X								
<b>Assessment of tolerability:</b>													
<b>Toxicity according to CTCAE criteria</b>			X	X	X	X	X	X	X	X	X	X	X

## **Allogeneic BM-MSC manufacturing**

BM was harvested from donor iliac crest, transferred to a bag containing 5 milliliters of Citrate Dextrose Solution, and immediately shipped in an isothermal box to the Cell Therapy Unit for manufacturing. The BM aspirate was directly seeded at  $5 \times 10^5$  nucleated cells/cm<sup>2</sup> in culture chambers with Minimal Essential Medium Alpha (MEM-alpha) (Macopharma, France), 5% human platelet lysate (HPL) (Centre de Transfusions des Armées (CTSA), France) and 2 IU/mL heparin (Medium 1) and placed in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Non adherent cells were discarded after 2 days of culture and medium was renewed twice a week until reaching >50% confluence (passage 0, P0). BM-MSC were then washed with Dulbecco's phosphate-buffered saline (DPBS), harvested using trypsin (Trypzean, Sigma-Aldrich) and further expanded in new culture chambers seeded at 4000 cells/cm<sup>2</sup> in MEM-alpha medium, 8% HPL and 2 IU/mL heparin (Medium 2). When reaching confluence, final product BM-MSC (passage 1, P1) were harvested as for P0, washed with DPBS, and re-suspended at a maximal concentration of  $2 \times 10^6$  BM-MSC/mL in NaCl 0.9% containing 0.5% of human albumin, according to the patient targeted dose, aiming at  $1 \times 10^6$  BM-MSC/kg for the first 10 patients and  $3 \times 10^6$  BM-MSC/kg for the 10 others).

## **BM-MSC quality controls**

### ***Immunophenotyping***

BM-MSC were incubated for 20 min at 4°C with monoclonal antibodies (anti-CD73 clone AD2, anti-CD90 clone 5E10, anti-CD105 clone 266, and anti-CD45 clone HI30, all from Becton Dickinson) conjugated to different fluorochromes (PE or FITC) or with appropriate isotype controls. Cell viability was measured by adding 7-Amino Actinomycin D (7-AAD) dye. Analyses were performed by flow cytometry (Navios, Beckman Coulter). Results were expressed as percentage of the total viable nucleated cells analysed.

### ***CFU-F assay***

BM aspirate were cultured in medium 1 (13000 nucleated cells/cm<sup>2</sup>), and P0 and P1 BM-MSC in medium 2 (8 cells/cm<sup>2</sup>) at 37°C and 5% CO<sub>2</sub>. After 10 days, medium was removed, cells were fixed and stained with May-Grunwald-Giemsa. Colonies containing  $\geq 50$  cells were counted.

### ***Growth capacity***

The population doubling (PD) for each passage was calculated using the formula:  $\text{Log}(\text{number of harvested cells} / \text{number of seeded cells}) / \text{Log}(2)$  with the number of CFU-F in BM used as the number of P0 seeded cells. Cumulative PD (cPD = PD at P0 + PD at P1) and Doubling Time (DT = culture time in hours between seeding and harvesting for each passage/PD) were calculated.

### ***Microbial testing***

Microbial testing was done by sample inoculation into a set of pre-filled BacT/ALERT bottles (Biomérieux, France) containing aerobic or anaerobic medium. Bottles were continuously agitated and monitored over a 10-day period in the BacT/ALERT 3D Systems. The presence of viable microorganisms was detected by measuring the CO<sub>2</sub> produced with a colorimetric sensor.

### ***Karyotype***

Karyotypes were performed at P1 using standard cytogenetic procedures. A minimum of 20 metaphases were fully analyzed by G-banding.

### ***Human Telomerase Reverse Transcriptase (hTERT) gene expression***

Total RNA was extracted using RNeasy Plus mini kit (Qiagen). cDNA synthesis was performed on 1 µg total RNA with the high-capacity cDNA reverse transcription kit (ThermoFischer). Real-time quantitative PCR (RT-PCR) was performed with *hTERT* Taqman Assay and the TaqMan Universal master mix (Applied Biosystems, CA). Gene expression was measured using the ABI Prism 7700 Sequence Detection System based on the  $\Delta\Delta\text{CT}$  calculation method with Glyceraldehyde 3-phosphatase deshydrogenase (*GAPDH*) as internal standard gene.

### ***Mixed Lymphocyte Reaction***

BM-MSC were cocultured at different ratios for 7 days with peripheral blood mononuclear cells (PBMC) pooled from 10 healthy donors and labeled with CellTrace Violet® (CTV). To determine the percentage of division (PDiv) of viable T cells, PBMC stained with 7-AAD/CD3/CD45 antibodies were analyzed using Navios (Beckman coulter) flow cytometer and FlowJo® v10 software. The percentage of inhibition (PI) for each ratio was calculated using  $\text{PI}_{\text{ratio } x} = (\text{Pdiv}_{\text{control}} - \text{Pdiv}_{\text{ratio } x}) / \text{Pdiv}_{\text{control}}$  and the corresponding area under the curve (AUC) as described.<sup>1</sup>

## **BM-MSC transcriptomic analysis**

Cryopreserved P1 BM-MSC from 17 donors were stabilized in RNAsStill (Real Biotech Corporation) prior to thawing. Cells were thawed on ice and recovered by centrifugation. Total RNA was extracted with the Aurum total RNA mini kit (Biorad) including an on-column DNase I digestion step. RNA was quantified using a BioDrop (Montreal Biotech) and the integrity evaluated on an Agilent TapeStation; the RNA integrity numbers were in average  $9.7 \pm 0.2$ . Ribosomal RNAs were depleted and libraries were prepared with the TruSeq stranded total RNA kit (Illumina), then multiplexed and sequenced on an Illumina NovaSeq 6000 Sprime flow cell in a paired-end 50bp configuration.

An average of 58 million sequence read pairs were obtained per sample and FastQC tool (Babraham Bioinformatics) was used to confirm the high sequence quality. The reads were mapped to the human hg38 genome reference assembly using HISAT2 v2.1.0 using the -k 1 --rna-strandness RF functions.<sup>2</sup> Gene expression was quantified by counting the number of

reads mapped to exon with the correct strand orientation using featureCounts.<sup>3</sup> Normalization and differential gene expression analyses were conducted using the edgeR Bioconductor package.<sup>4</sup> To avoid sex confounding effects, the Y chromosome genes were excluded as well as the X-inactivation gene *XIST*, and sex was included as a co-variate. Genes with an expression levels  $\geq 5$  counts per million reads (CPM) in at least 5 of the samples were retained and differential gene expression analysis was performed using the glmQLFTest by comparing donor MSC based on the best patient clinical response at three and six months post-infusion. For data visualization in IGV genome browser,<sup>5</sup> bigwig scaled per million reads mapping onto gene exons were generated using a succession of genomeCoverageBed and wigToBigWig tools.

### **Patient immune monitoring**

Circulating immune cell subsets were quantified by flow cytometry before, one month, and three months after allogeneic BM-MSCT treatment on fresh heparinized blood samples. Briefly, PBMC were obtained after Ficoll density gradient and stained with fluorochrome-conjugated monoclonal antibodies targeting CD3 (SK7 and UCHT1 clones), CD4 (SK3 clone), CD8 (SK1 clone), CD14 (M $\phi$ P9 clone), CD16 (3G8 and B73.1 clones), CD19 (SJ25C1 clone), CD45 (HI30 and 2D1 clones), CD56 (NCAM16.2), CD335/NKp46 (9E2 clone), and HLA-DR (G46-6 clone). Lymphocyte subpopulations were analyzed as CD45<sup>pos</sup>CD3<sup>pos</sup> T lymphocytes, CD45<sup>pos</sup>CD19<sup>pos</sup> B lymphocytes, and NK cells defined as CD45<sup>pos</sup>CD3<sup>neg</sup>CD19<sup>neg</sup> cells expressing CD16 and/or CD56. Monocytes were analyzed on CD45<sup>pos</sup>CD3<sup>neg</sup>CD19<sup>neg</sup>CD335<sup>neg</sup>HLA-DR<sup>pos</sup> cells as CD14<sup>pos/low</sup> cells expressing variable levels of CD16. Absolute counts were determined using count beads (Biolegend) with BD Multitest (BD Biosciences).

### **HLA genotyping.**

DNA was extracted from anticoagulated whole blood, PBMC, or granulocytes isolated by Ficoll gradient centrifugation (Sigma-Aldrich) using the EZ1 Advanced XL instrument (Qiagen, France). All HLA-A, B, C, DRB1, DQA1, DQB1, DPA1, DPB1 genotyping for the recipients and their donors were performed to achieve a resolution level of the second field according to the WHO HLA nomenclature ([www.HLANomenclature@hla.alleles.org](http://www.HLANomenclature@hla.alleles.org)) using the available method at time of blood sampling (PCR-Sequence Specific Oligonucleotides, One Lambda; PCR- Sequence Specific Primers (PCR-SSP), Olerup; Next Generation Sequencing, Gendx). At this resolution level, all alleles with the same name encode the same specific HLA molecule.

### **Anti-HLA Class I and II antibody detection and characterization**

Anti-HLA antibodies were searched before, one, and three months after BM-MSCT infusion using Luminex Single Antigen (One Lambda). Mean fluorescence intensity measured on a LABScan 200 platform were considered positive with a cutoff of 2000, as per the Agence de la Biomédecine recommendation. If the recipient had antibodies against HLA-DR51, DR52, or DR53, the donor was typed by PCR-SSP for the HLA-DRB5, DRB3, or DRB4 genes, which respectively code for these three specific HLA molecules.

### **Microchimerism.**

The presence of donor chimerism was searched before and one month after BM-MSCT infusion. Non-shared donor/recipient HLA-specific quantitative PCR assays were performed as described<sup>6</sup>, with 0.005% sensitivity detection. Results were expressed in genome equivalent of donor cells per million of host cells (gEq/10<sup>6</sup>).

### **Bayesian analysis methodology**

Prior density has been chosen in the family of non-informative Beta density B(0.2,0.8). Bayesian stopping rules were calculated after the inclusion of the first ten patients at 1x10<sup>6</sup>/kg BM-MSCT, to eventually decide stopping the inclusions and/or to increase (3x10<sup>6</sup>/kg) or decrease (0.5x10<sup>6</sup>/kg) the BM-MSCT target dose. Criteria were defined as follows: criterion 1, posterior probability that the rate of intolerance  $\pi$  is greater than 20% (the threshold of intolerance considered), criterion 2, posterior probability that the rate of intolerance  $\pi$  is less than 20%. Rules were the following: 1) If criterion 1 > 0.70: stop inclusions at 1x10<sup>6</sup>/kg and reduce dose to 0.5x10<sup>6</sup>/kg for the next ten patients 2) If criterion 2 > 0.70 stop inclusions at 1x10<sup>6</sup>/kg and increase dose to 3x10<sup>6</sup>/kg for the next ten patients. If none of the criteria is filled, the next ten patients will be included at the same starting dose. Probabilities of response at M3 and M6 were analyzed as the probability of intolerance but with Beta density B(0.5, 0.5) used as prior densities.

**Supplementary Table 1: List of soluble factors measured by Luminex technology in plasma obtained before, three and six months after allogeneic BM-MSC infusion in 20 SSc patients.**

Plasma were stored at -80°C until use for quantification of 51 soluble factors by Luminex technology.

Soluble Factors	Magnetic Bead Region	Gene symbol	Manufacturer	Reference
Basic FGF	44	FGF2	Biorad	Screening 48-Plex panel (catalog #12007283)
CTACK	72	CCL27	Biorad	Screening 48-Plex panel (catalog #12007283)
Eotaxin	43	CCL11	Biorad	Screening 48-Plex panel (catalog #12007283)
G-CSF	57	CSF3	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>GM-CSF*</i>	34	GM-CSF	Biorad	Screening 48-Plex panel (catalog #12007283)
GRO- $\alpha$	61	CXCL1	Biorad	Screening 48-Plex panel (catalog #12007283)
HGF	62	HGF	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IFN-<math>\alpha</math>2*</i>	20	IFNA2	Biorad	Screening 48-Plex panel (catalog #12007283)
IFN- $\gamma$	21	IFNG	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-10	56	IL10	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-12 (p40)	28	IL-12 (p40)	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-12 (p70)*</i>	75	IL-12 (p70)	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-13*</i>	51	IL13	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-15*</i>	73	IL15	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-16	27	IL16	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-17*</i>	76	IL17	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-18	42	IL18	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-1ra	25	IL1RN	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-1 $\alpha$	63	IL1A	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-1 $\beta$	39	IL1B	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-2	38	IL2	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-2R $\alpha$	13	IL2RA (13)	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-3	64	IL3	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-4*</i>	52	IL4	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-5*</i>	33	IL5	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-6	19	IL6	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>IL-7*</i>	74	IL7	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-8	54	IL8	Biorad	Screening 48-Plex panel (catalog #12007283)
IL-9	77	IL9	Biorad	Screening 48-Plex panel (catalog #12007283)
IP-10	48	CXCL10	Biorad	Screening 48-Plex panel (catalog #12007283)
LIF	29	LIF	Biorad	Screening 48-Plex panel (catalog #12007283)
MCP-1	53	CCL2	Biorad	Screening 48-Plex panel (catalog #12007283)
<i>MCP-3*</i>	26	CCL7	Biorad	Screening 48-Plex panel (catalog #12007283)
M-CSF	67	CSF1	Biorad	Screening 48-Plex panel (catalog #12007283)
MIF	35	MIF	Biorad	Screening 48-Plex panel (catalog #12007283)
MIG	14	CXCL9	Biorad	Screening 48-Plex panel (catalog #12007283)
MIP-1 $\alpha$	55	CCL3	Biorad	Screening 48-Plex panel (catalog #12007283)
MIP-1 $\beta$	18	CCL4	Biorad	Screening 48-Plex panel (catalog #12007283)
NGAL	14	LCN2	ThermoFisher	ProcartaPlex (Catalog #PPX-02-MXZTE9E)
PDGF-BB	47	PDGFB	Biorad	Screening 48-Plex panel (catalog #12007283)
RANTES	37	CCL5	Biorad	Screening 48-Plex panel (catalog #12007283)

SAA	26	SAA1	ThermoFisher	ProcartaPlex (Catalog #PPX-02-MXZTE9E)
SCF	65	KITLG	Biorad	Screening 48-Plex panel (catalog #12007283)
SCGF- $\beta$	78	SCGF-B	Biorad	Screening 48-Plex panel (catalog #12007283)
SDF-1 $\alpha$	22	CXCL12	Biorad	Screening 48-Plex panel (catalog #12007283)
TGF $\beta$ 1	13	TGFB1	Biorad	Bioplex pro TGF $\beta$ 1 set (catalog #171V4001M + #171304090M + #171X40001)
TNF- $\alpha$	36	TNF	Biorad	Screening 48-Plex panel (catalog #12007283)
TNF- $\beta$	30	LTA	Biorad	Screening 48-Plex panel (catalog #12007283)
TRAIL	66	TNFSF10	Biorad	Screening 48-Plex panel (catalog #12007283)
VEGF	45	VEGFA	Biorad	Screening 48-Plex panel (catalog #12007283)
$\beta$ -NGF*	46	NGF	Biorad	Screening 48-Plex panel (catalog #12007283)

\* below the quantification threshold

## Supplementary Table 2: List of soluble factors measured by Luminex in BM-MSC supernatants

Soluble Factors	Magnetic Bead Region	Gene symbol	Manufacturer	Reference
G-CSF*	20	CSF3	Merck / Millipore	HCYTA-60K_07
IFN-a2	25	IFNA2	Merck / Millipore	HCYTA-60K_07
IL-7*	37	IL7	Merck / Millipore	HCYTA-60K_07
IP-10	57	CXCL10	Merck / Millipore	HCYTA-60K_07
MCP-1	61	CCL2	Merck / Millipore	HCYTA-60K_07
MIG	65	CXCL9	Merck / Millipore	HCYTA-60K_07
VEGF*	78	VEGFA	Merck / Millipore	HCYTA-60K_07

\* below the quantification threshold



**Supplementary Table 3: Detailed list of severe adverse events (SAE), as defined by grade  $\geq 3$  adverse events according to the NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0) and reported from time of allogeneic bone marrow-mesenchymal stromal cell (BM- MSC) infusion until last follow-up for all treated patients (n=20) with severe diffuse Systemic Sclerosis.**

<b>Organs (according the CTCAE v.5.0)</b>	<b>Patient ID</b>	<b>Sex</b>	<b>Severe Adverse events</b>	<b>Time of onset after infusion (months)</b>	<b>Maximal grade (CTCAE v5.0)</b>
<b>Cardiac disorders</b>	#2	M	Right auricular thrombus	11.7	3
	#4	F	Sick sinus syndrome	2.8	3
			Ventricular tachycardia	13.2	3
			Ventricular arrhythmia	16.0	3
			Ventricular arrhythmia	15.3	3
			Tachycardia	18.7	3
			Sinus Tachycardia	20.7	3
	#7	F	Arterial hypertension	12.0	3
	#11	F	Atrioventricular block complete	7.3	3
	#13	F	Pacemaker implantation	1.4	3
SSc-specific arrhythmogenic cardiomyopathy			16.8	3	
#18	M	Cardiac arrest and resuscitation	8.3	4	
<b>Eye disorders</b>	#8	M	Cataract	12.8	3
<b>Gastrointestinal disorders</b>	#2	M	Chronic intestinal pseudo-obstruction (flare-up )	9.7	3
	#3	M	Esophageal foreign body (fish bone)	8.2	3
	#13	F	Anorexia / malnutrition	15.8	3
			Chronic intestinal pseudo-obstruction	15.8	3
#14	F	Chronic intestinal pseudo-obstruction (flare-up )	8.0	3	
<b>Infections and infestations</b>	#3	M	Viral lung infection	23.1	3
	#4	F	Viral lung infection (influenza)	9.4	3
			Kidney infection	16.4	3
			Viral lung infection/asthenia (influenza)	22.7	3
	#15	F	Urinary infection	8.0	3
	#18	M	Prostate infection	0.8	3
Prostate infection			13.8	3	
<b>Injury, poisoning and procedural complications</b>	#16	F	Fracture (femoral neck)	22.3	3
<b>Investigations</b>	#5	F	Anemia	5.6	3
<b>Neoplasms benign, malignant and unspecified</b>	#16	F	Basal cell carcinoma (left cheek)	5.3	3
<b>Renal and urinary disorders</b>	#12	F	Renal crisis and thrombotic microangiopathy	1.4	3
<b>Respiratory, thoracic and mediastinal disorders</b>	#18	M	Pleural effusion	6.8	3
<b>Skin and subcutaneous tissue disorders</b>	#4	F	Skin ulceration (right heel)	7.6	3
	#9	M	Skin ulceration (right leg)	8.7	3
	#13	F	Skin ulceration (subcutaneous calcification)	11.8	3
	#15	F	Skin ulceration (4th finger of left hand)	19.2	3
<b>Vascular disorders</b>	#12	F	Blue toe syndrome	22.6	3

**Supplementary Table 4: Detailed list of non-severe adverse events (AE), as defined by grade  $\leq 2$  adverse events according to the NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0) and reported from time of allogeneic bone marrow-mesenchymal stromal cell (BM- MSC) infusion until last follow-up for all treated patients (n=20) with severe diffuse Systemic Sclerosis.**

Treatment-related AE (n= 3 in two patients) are presented in **bold**.

Organs (according the CTCAE v.5.0)	Patient ID	Sex	Adverse events	Time of onset after infusion (Months)	Maximal grade (CTCAE v5.0)
Cardiac disorders	#01	F	Mitral valve disease	24.4	1
			Tricuspid valve disease	15.4	2
			Aortic valve disease	21.3	1
	#02	M	Tricuspid valve disease	3.6	2
			Right ventricular dysfunction	11.7	1
			Mitral valve disease	3.6	1
	#04	F	Limb oedema	10.7	2
			Others (right auricular thrombus)	2.1	2
			Ventricular arrhythmia	20.7	2
	#06	F	Tricuspid valve disease	8.6	2
			Palpitations	18.9	1
	#07	F	Mitral valve disease	6.0	1
	#08	M	Mitral valve disease	5.7	1
	#10	F	Right ventricular dysfunction	6.3	1
Right ventricular dysfunction			1.3	2	
Mitral valve disease			9.1	2	
#12	F	Palpitations	17.3	1	
#12	F	Arterial hypertension despite treatment	0.5	2	
Ear and labyrinth disorders	#10	F	Other (inflammatory nasal dryness)	21.2	1
Endocrine disorders	#07	F	Hypothyroidism	3.1	2
Eye disorders	#08	M	Dry eye	23.8	2
	#10	F	Other (chalazion)	6.3	2
	#13	F	Other (eyelid inflammation)	2.8	1
	#17	M	Other (chalazion)	2.9	2
Gastrointestinal disorders	#01	F	<b>Nausea</b>	<b>0.0</b>	<b>1</b>
			Other (gastroparesis)	24.4	1
	#02	M	Other (gastrointestinal pain)	9.7	1
			Vomiting	9.7	2
			Diarrhea	3.6	2
			Fecal incontinence	21.7	2
			Other (stercoral colitis)	3.6	2
	#03	M	Vomiting	5.7	1
			Vomiting	14.9	2
	#05	F	Vomiting	20.8	1
	#06	F	Other (gastritis)	6.6	2
	#07	F	Nausea	8.8	1
	#07	F	Other (odynophagia)	8.7	1
	#08	M	Periodontal disease	23.8	2
			Periodontal disease	12.9	2
	#10	F	Fecal incontinence	17.3	1
			Other (constipation)	12.3	2
			Diarrhea	15.3	2
	#13	F	Chronic intestinal pseudo-obstruction	2.8	2
#14	F	Periodontal disease	13.1	2	
#15	F	Other (undefined digestive disorders)	7.1	1	
		Other (weight gain)	1.4	1	
#17	M	Other (molar fracture)	0.9	1	
#18	M	Chronic intestinal pseudo-obstruction	6.8	2	
		Diarrhea	18.1	1	
General disorders	#01	F	<b>Other (asthenia)</b>	<b>0.0</b>	<b>2</b>
			Other (0.5 increase in SHAQ score)	12.3	2
	#02	M	Fatigue	10.7	1
	#03	M	Non cardiac chest pain	18.9	1
			Other (flu like symptoms)	10.9	2
	#04	F	Non cardiac chest pain	19.1	1
			Flu-like symptoms	0.5	1
	#07	F	Fever	2.7	1
			Pain	5.7	2
#08	M	Flu-like symptoms	14.8	2	
#14	F	Fatigue	1.1	1	

	#15	F	Pain	3.0	1
<b>Hepatobiliary disorders</b>	#04	F	Other (hepatic steatosis)	9.0	1
<b>Infections and infestations</b>	#03		Skin infection	23.0	2
			Skin infection	9.1	2
			Skin infection	4.9	2
	#04	F	Urinary infection	13.6	2
	#07	F	Lung infection	24.7	2
			Urinary infection	5.7	2
			Other (conjunctivitis)	14.7	2
			Other (rhinitis infective)	24.7	2
			Other (esophageal infection)	15.7	2
			Other (sinusitis)	15.7	2
			Skin infection	16.7	2
	#08	M	Lung infection	24.8	2
			Lung infection	11.8	2
			Other (pharyngitis)	3.7	2
			Skin infection	11.8	2
	#09	M	Skin infection	8.7	2
			Prostate infection	23.6	2
	#10	F	Other (dental abscess)	7.9	1
Other (sinusitis)			19.3	2	
#12	F	Other (rhinitis infective)	9.0	1	
#13	F	Other (abdominal infection)	8.9	1	
#16	F	Skin infection	5.3	2	
#20	M	Skin infection	4.1	2	
<b>Investigations</b>	#02	M	Weight loss	21.7	1
	#04	F	Anemia	20.7	1
			Anemia	22.7	1
	#08	M	Lymphopenia	9.3	2
	#07	F	Weight loss	2.7	1
	#09	M	Anemia	17.2	1
	#10	F	Other (inflammatory syndrome)	17.3	1
	#12	F	Weight loss	9.4	1
			Anemia	12.5	2
#13	F	Anemia	3.0	1	
#15	F	Anemia	1.4	1	
<b>Metabolism and nutrition disorders</b>	#07	F	Anorexia	8.7	1
	#08	M	Other (hypogammaglobulinemia)	9.8	1
	#10	F	Other (vitamin D deficiency)	21.3	1
	#12	F	Other (folate deficiency)	10.4	2
<b>Musculoskeletal and connective tissue disorders</b>	#01	F	Other (flank pain)	18.3	1
	#02	M	Arthralgia	21.3	2
			Arthralgia	11.7	2
			Arthralgia	11.7	2
	#03	M	Other (post traumatic right paravertebral pain)	6.9	2
	#05	F	Other (right psoas tear after stretching)	8.5	1
	#06	F	Myalgia	6.9	2
			Arthralgia	5.9	2
	#07	F	Arthralgia	5.2	2
			Other (dorsal interscapular pain during deep inspiration/blocking sensation)	2.7	2
	#08	M	Arthralgia	3.1	2
			Myalgia	18.0	2
	#09	M	Other (tendon friction rub)	11.6	1
	#10	F	Other (left biceps tendinitis pain)	17.3	2
			Myalgia	6.3	2
			Arthralgia	12.3	2
	#15	F	Myalgia	0.4	2
			Other (tendinitis of the right buttock)	5.9	2
Other (tendinitis- right wrist)			5.4	2	
Arthralgia			3.4	2	
#17	M	Arthralgia	4.9	1	
#18	M	Other (back pain)	9.8	2	
		Other (osteoporosis)	6.8	1	
<b>Neoplasms benign- malignant and unspecified</b>	#05	F	Gastric fundic polyps	6.5	1
	#15	F	Left submandibular gland hypertrophy	0.5	1
<b>Nervous system disorders</b>	#01	F	Voice alteration	3.4	2
	#02	M	Anxiety	9.0	1
	#03	M	Headache	7.4	2
	#04	F	Paresthesia	18.2	1
	#08	M	Anxiety	14.8	1
Other (sleep disorders)			14.8	2	

			Radiculitis	23.8	2
	#10	F	Dysesthesia	20.4	2
			Other (frequent discomfort)	18.3	2
			Anxiety	18.3	1
	#13	F	Headaches	1.8	2
	#15	F	Syncope	11.9	2
<b>Renal and urinary disorders</b>	#02	M	Urinary incontinence	7.3	1
			Proteinuria	7.3	2
<b>Reproductive system</b>	#08	M	Erectile dysfunction	17.7	2
	#09	M	Erectile dysfunction	20.7	1
<b>Respiratory· thoracic and mediastinal disorders</b>	#01	F	Dyspnea	0.3	2
			Cough	3.4	2
			Other (allergic rhinitis)	4.3	1
			Cough	15.4	2
			Pulmonary hypertension	18.3	2
			Other (epistaxis)	21.3	1
	#02	M	Dyspnea	3.6	2
			Dyspnea	21.7	2
			Pulmonary hypertension	18	2
			Voice alteration	18.1	1
	#03	M	Cough	0.9	1
	#04	F	Other (nasal congestion)	0.1	1
			Dyspnea	5.9	1
	#06	F	Dyspnea	8.8	1
			Other (allergic rhinitis)	14.9	1
			Dyspnea	24.8	1
	#07	F	Voice alteration	14.7	1
			Voice alteration	14.7	1
Dyspnea			0.7	2	
#09	M	Dyspnea	23.6	1	
		Other (lung emphysema)	11.6	1	
#10	F	Dyspnea	3.3	1	
#15	F	Dyspnea	8.9	1	
#18	M	Other (pneumonitis)	2.8	1	
<b>Skin and subcutaneous tissue disorders</b>	#02	M	Skin ulceration	8.7	2
			Skin ulceration	8.7	2
			Skin ulceration	24.7	2
			Other (skin hypopigmentation)	11.7	2
			Other (pruritus)	11.7	2
			Skin ulceration	21.7	2
	#03	M	Other (pilodinal cyst)	2.1	2
	#04	F	Other (skin hyperpigmentation)	6.6	2
			Other (skin hypopigmentation)	6.6	2
	#07	F	Other (subcutaneous nodule)	20.7	2
			Other (skin erythema)	22.7	1
	#09	M	Skin ulceration	5.2	2
			Skin ulceration	8.9	2
			Skin ulceration	11.6	2
			Other (ochre dermatitis)	11.6	1
			Skin ulceration	1.6	2
			Other (telangiectasia)	12.6	1
	#10	F	Skin ulceration	3.2	1
Skin ulceration			10.3	2	
#13	F	Skin ulceration	12.0	2	
#15	F	Other (hyperpigmentation)	7.1	1	
		Skin ulceration	0.5	1	
<b>Vascular disorders</b>	#01	F	Lymphocele	15.4	1
	#02	M	Thrombophlebitis	24.7	2
			Other (venous insufficiency)	17.7	1
	#05	F	<b>Flushing</b>	<b>0.0</b>	<b>1</b>
	#06	F	Other (asymptomatic intra cerebral aneurism)	6.9	1
	#11	F	Superficial thrombophlebitis	4.1	2

**Supplementary Table 5. Quality controls of allogeneic bone marrow-mesenchymal stromal cell (BM-MS) products released for infusion to the patients (n=20) with severe Systemic Sclerosis**

	Results	Specification	Out of specification (OOS)	
			n	Details of OOS
Delivered cell dose (MSC x 10 <sup>6</sup> /kg)				
- Patient group 1·0·10 <sup>6</sup> /kg (n=10)	1·0 [1·0 ; 1·0] (0·6 ; 1·0)	1·0·10 <sup>6</sup> /kg	2/10*	(#7) 0·6·10 <sup>6</sup> /kg (#9) 0·9·10 <sup>6</sup> /kg
- Patient group 3·0·10 <sup>6</sup> /kg (n=10)	3·0 [2·2 ; 3·0] (1·0 ; 3·0)	3·0·10 <sup>6</sup> /kg	3/10	(#13) 1·0·10 <sup>6</sup> /kg (#15) 2·3·10 <sup>6</sup> /kg (#16) 1·7·10 <sup>6</sup> /kg
Cell viability (%)	95·1 [90·2; 96·9] (65·4 ;98·8)	≥ 85·0%	1/20	(#7) 65·4%
Immunophenotyping				
CD90 (%)	99·7 [98·9;99·9] (95·3;100·0)	≥ 90·0%	0/20	
CD73 (%)	99·2 [96·3;99·8] (91·5;100·0)	≥ 90·0%		
CD105 (%)	98·2 [94·2;99·8] (87·9;100·0)	≥ 80·0%		
CD45 (%)	0·07 [0;0·5] (0;1·9)	≤ 5·0%		
Microbiology	No growth (20/20)	No growth	0/20	

Results (n=20) are expressed as median [IQR] (min; max). Out-of-specification (OOS) represents the number of batches (n) released at the manufacturer responsibility with a result non-compliant with the defined specifications.

(#) corresponding donor number.

- (#5) For patient 5 donor, after uneventful BM harvesting and shipping, BM-MS were compliant until day 18, when diffuse fibrin deposit appeared on culture which had to be discarded. A second manufacturing process was successfully performed after thawing the cryopreserved BM sample from the same donor.

- (#7) For patient 7 donor, the BM-MS reached over-confluence and detached from plastic before harvest, which resulted in 65·4% final product cell viability and a lower than expected target dose administered to the patient (0·6x10<sup>6</sup> BM-MS/kg).

- (#9) For patient 9 donor, a first BM sample was harvested with anticoagulant from his 36-year-old healthy brother. No MS were obtained after 10 days culture. A second donor, his 31-year-old wife was selected for a new and second bone-marrow harvesting, which BM-sample culture provided 0·92x10<sup>6</sup> BM-MS /kg bodyweight.

### Supplementary Table 6: Allogeneic bone marrow-mesenchymal stromal cell (BM-MSC) production process.

Data are given for initial (BM), intermediate (Passage 0 (P0)), and final (Passage 1 (P1)) cell products.

Patient ID	Donor age (years) / gender (F/M)	Bone Marrow (BM)				BM-MSC P0				BM-MSC P1													
		Initial Product				Intermediate Product				Final Product													
		Total collection volume (mL)	Nucleated cells count (10 <sup>6</sup> /mL)	Nucleated cells viability (%)	CFU-F/10 <sup>6</sup> nucleated cells	CFU-F/10 <sup>6</sup> MSC	Expansion time from BM seeding (days)	Number of population doubling	Doubling Time (hours)	CFU-F/10 <sup>6</sup> MSC	Expansion time from BM seeding (days)	Number of population doubling	Number of cumulated population doubling	Doubling Time (hours)	MSC viability (%)	CD45+ (%)	CD90+ (%)	CD73+ (%)	CD105+ (%)	Released dose (10 <sup>6</sup> MSC/kg)	Sterility Control	Karyotype formula	hTERT expression
#1	52 / F	40	5.7	91.4	63.6	75000	13.0	12.8	24.4	27500	21.0	5.0	17.8	38.4	90.1	0.1	97.1	91.5	90.6	1.0	No growth	46, XX [36]	Negative
#2	38 / M	26	22.3	85.0	174.2	330000	12.0	14.1	20.4	172500	18.0	4.1	18.2	35.4	96.4	0.2	99.8	99.8	99.8	1.0	No growth	46, XY [27]	Negative
#3	44 / F	19	17.3	93.1	62.1	67500	14.0	12.9	26.0	122500	19.0	2.9	15.8	41.4	96.4	0.0	99.4	93.5	94.5	1.0	No growth	47, XX, +5 [22]; 49, XX, +2, +5, +6 [1]; 46, XX [8]	Negative
#4	24 / M	25	21.5	78.9	97.0	180000	14.0	12.0	28.0	75000	20.0	4.1	16.1	35.0	98.0	0.1	100.0	100.0	100.0	1.0	No growth	46, XY [33]	Negative
#5	49 / F	BM thawing	NA	NA	30.3	ND	12.0	13.1	22.0	157500	20.0	3.2	16.4	60.0	93.7	1.9	97.9	94.6	87.9	1.0	No growth	46, XX [31]	Negative
#6	51 / F	62	10.8	87.1	209.1	240000	12.0	11.7	24.6	27500	18.0	3.1	14.8	45.7	89.6	0.5	99.7	99.8	99.8	1.0	No growth	46, XX [31]	Negative
#7	31 / M	68	24.1	94.0	16.7	270000	12.0	14.6	19.7	2500	18.0	ND	ND	ND	65.4	1.6	98.6	96.2	94.0	0.6	No growth	46, XY [30]	Negative
#8	45 / M	40	25.6	95.1	78.8	160000	14.0	12.6	26.7	167500	19.0	4.0	16.6	30.0	90.4	0.0	98.9	96.5	96.6	1.0	No growth	46, XY [31]	Negative
#9	31 / F	64	6.3	93.0	15.2	160000	14.0	13.0	25.8	207500	20.0	3.3	16.4	43.6	94.2	0.0	98.8	99.1	99.1	0.9	No growth	46, XX [32]	ND
#10	19 / M	18	31.8	94.6	136.4	255000	14.0	13.4	25.1	187500	19.0	5.1	18.4	23.5	96.9	0.0	99.9	99.8	99.9	1.0	No growth	47, XY, +8 [2] ; 46, XY [28]	Negative
#11	36 / F	100	12.0	90.3	55.3	102500	13.0	13.0	24.0	112500	19.0	3.5	16.5	40.6	89.1	0.3	99.9	99.0	94.1	3.0	No growth	46, XX [30]	Negative

#12	40 / M	85	24.2	91.0	9.1	150000	14.0	14.9	22.6	122500	21.0	1.9	16.7	88.4	96.8	0.0	99.2	97.9	97.0	1.0	No growth	46, XY [36]	Negative
#13	48 / M	110	20.4	86.5	30.3	140000	13.0	13.9	22.4	90000	19.0	3.3	17.2	43.3	89.8	0.8	100.0	99.8	98.8	3.0	No growth	47, XY, +5 [2]; 46, XY [28]	Negative
#14	44 / M	85	10.5	95.9	18.2	ND	14.0	11.8	28.5	ND	21.0	3.7	15.5	45.4	97.4	0.0	99.6	99.4	97.9	2.3	No growth	46, XY [9]	Negative
#15	35 / F	106	4.9	91.7	6.1	ND	13.0	12.3	25.4	83750	21.0	5.6	17.9	34.4	92.2	0.1	99.9	99.9	99.7	1.7	No growth	46, XX [31]	Negative
#16	26 / F	94	10.0	95.4	9.1	ND	14.0	15.1	22.3	30000	21.0	3.6	18.6	46.7	98.8	0.0	99.7	99.3	98.4	3.0	No growth	46, XX [30]	Negative
#17	33 / M	68	15.8	97.6	22.7	47500	14.0	15.0	22.4	52500	19.0	3.8	18.8	31.6	97.9	0.0	99.8	97.4	95.2	3.0	No growth	46, XX [30]	Negative
#18	51 / F	87	12.3	95.4	15.2	125000	13.0	14.3	21.8	97500	19.0	3.8	18.0	37.9	95.1	0.0	95.3	94.7	91.2	3.0	No growth	46, XX [30]	Negative
#19	47 / F	75	10.3	93.2	34.8	67500	13.0	12.0	26.0	112500	19.0	3.8	15.8	38.3	95.1	0.0	100.0	99.8	99.7	3.0	No growth	46, XX[30]	Negative
#20	27 / F	59	17.3	90.1	79.5	142500	13.0	13.0	24.0	93750	19.0	3.9	16.9	37.1	96.3	0.6	99.9	100.0	100.0	3.0	No growth	46, XX [31]	Negative
Min	19	18	4.9	78.9	6.1	47500	12.0	11.7	19.7	2500	18.0	1.9	14.8	23.5	65.4	0.0	95.3	91.5	87.9	0.6			
Q1 (IQR)	31	40	10.3	90.1	15.6	81875	13.0	12.4	22.3	52500	19.0	3.3	16.3	35.2	90.2	0.0	98.8	96.3	94.2	1.0			
Median	39	68	15.8	93.0	32.6	146250	13.0	13.0	24.2	97500	19.0	3.8	16.7	38.4	95.1	0.1	99.7	99.2	98.2	1.0			
Q3 (IQR)	48	87	22.3	95.1	79.3	225000	14.0	14.3	26.0	157500	20.8	4.0	18.0	44.5	96.9	0.5	99.9	99.8	99.8	3.0			
Max	52	110	31.8	97.6	209.0	330000	14.0	15.1	28.5	207500	21.0	5.6	18.8	88.4	98.8	1.9	100.0	100.0	100.0	3.0			
Mean	39	64	16.0	91.5	58.2	157031	13.3	13.3	24.1	102237	19.5	3.8	17.0	41.9	93.0	0.3	99.2	97.9	96.7	1.8			
Standard Deviation	10	29	7.6	4.6	57.4	81247	0.8	1.1	2.4	58581	1.1	0.8	1.1	13.6	7.2	0.5	1.2	2.6	3.6	1.0			

NA: not applicable; ND: not done

**Supplementary Table 7. Evolution of clinical parameters according to time after allogeneic bone marrow-mesenchymal stromal cells**

Parameters	M0		M3		M6		M12	
	No.	Median [IQR]	No.	Median [IQR]	No.	Median [IQR]	No.	Median [IQR]
Modified Rodnan Skin Score	20	23 [21 ; 29]	20	15 [13 ; 20]	19	18 [13 ; 23]	18	19 [12 ;26]
FVC % of predicted	19	69 [66 ; 80]	18	70 [66 ; 76]	18	72 [68 ; 78]	16	72 [68 ; 78]
DLCO % of predicted	19	41 [34 ; 50]	17	39 [28 ; 45]	18	40 [33 ; 46]	15	36 [31 ; 47]
LVEF (%)	20	64 [61 ; 73]			18	61 [59 ; 66]	15	64 [60 ; 69]
Serum creatinine (N < 120 µmol/l)	20	54 [45 ; 73]	20	53 [47 ; 76]	20	56 [45 ; 74]	19	55 [43 ; 72]
HAQ-DI	20	1.5 [0.6 ; 1.6]	20	1.4 [0.5 ; 1.8]	19	1.6 [0.6 ; 2.0]	17	1.5 [0.9 ; 1.9]
VAS HAQ score	19	19 [2 ; 41]	18	20 [10 ; 40]	18	26 [11 ; 45]	16	17 [6 ; 31]
EQ5D								
Index-based utility score	19	0.69 [0.48 ; 0.78]	17	0.66 [0.48;0.78]	16	0.59 [0.53 ; 0.76]	16	0.64 [0.49 ; 0.78]
VAS EQ5D score	20	60 [45 ; 70]	15	70 [55 ; 75]	16	60 [48 ; 71]	16	63 [50 ; 72]
SF36 global	20	44 [28 ; 52]	19	48 [30 ; 72]	18	31 [24 ; 56]	18	54 [31 ; 71]
SF36 physical component	20	29 [20 ; 47]	19	38 [27 ; 67]	18	32 [21 ; 50]	18	51 [33 ; 66]
SF36 mental component	20	56 [37 ; 69]	19	47 [32 ; 86]	18	33 [23 ; 61]	18	60 [32 ; 78]

**(BM-MSC) infusion in patients (n=20) with severe Systemic Sclerosis**

DLCO: diffusing capacity of the lungs for carbon monoxide, EQ5D: EuroQol 5 dimensions, FVC: forced vital capacity, HAQ-DI: health assessment questionnaire - disability index, LVEF: left ventricular ejection fraction, SF-36: 36-Item Short Form General Health Survey, VAS: visual analog scale



**Supplementary Table 8: Donor Specific Antibodies (DSA) in patients (n=20) with severe Systemic Sclerosis (SSc) before, one (M1), and three (M3) months after allogeneic bone-marrow mesenchymal stromal cells (BM-MSc) infusion**

The maximum median fluorescence intensity was defined by the highest ranked donor specific bead. Before BM-MSc infusion, 3 patients (15%) had DSA, which disappeared one month later (patient #14) or remained positive until three months (patients #12, and #19). Two patients **in bold** (10%) developed *de novo* DSA at one month, still present at three months (patients #1, and #11).

Patient ID	D0		M1		M3	
	HLA Class I DSA	HLA Class II DSA	HLA Class I DSA	HLA Class II DSA	HLA Class I DSA	HLA Class II DSA
#1	/	/	<b>2329</b>	/	<b>2716</b>	/
#2	/	/	/	/	/	/
#3	/	/	/	/	/	/
#4	/	/	/	/	/	/
#5	/	/	/	/	/	/
#6	/	/	/	/	/	/
#7	/	/	/	/	/	/
#8	/	/	/	/	/	/
#9	/	/	/	/	/	/
#10	/	/	/	/	/	/
#11	/	/	<b>6692</b>	/	<b>3997</b>	/
#12	7773	/	4332	/	3771	/
#13	/	/	/	/	/	/
#14	/	2702	/	/	/	/
#15	/	/	/	/	/	/
#16	/	/	/	/	/	/
#17	/	/	/	/	/	/
#18	/	/	/	/	/	/
#19	3626	/	4523	/	5976	/
#20	/	/	/	/	/	/

**Supplementary Table 9: Donor microchimerism before and one month after allogeneic bone-marrow mesenchymal stromal cells (BM-MSK) infusion in patients with severe Systemic Sclerosis**

Samples from whole blood, peripheral blood mononuclear cells, and granulocytes from the 16 out of the 20 patients informative for non-shared donor HLA analysis have been tested prior to and after transplantation for donor microchimerism. Detection and quantification are based on Human Leucocyte Antigen (HLA) allele differences between donor and recipient and is realized by HLA-specific PCR. Two patients (#4, and #8) could be tested with two different detectors as their HLA genotyping was different from donors for both HLA-DRB1 alleles. Results are expressed in genome equivalent of donor cells per million of host cells (gEq/10<sup>6</sup>). Thirteen patients (81%) had undetectable donor chimerism in any blood compartment before and after BM-MSK infusion. Low levels of naturally acquired chimerism were detected in three patients (*in bold #4, #8 and #10, see explanatory details in supplementary Figure 1*)

Patient ID	HLA-QPCR Detector	Before allogeneic BM-MSK infusion						One month after allogeneic BM-MSK infusion					
		Whole blood		Peripheral blood mononuclear cells		Granulocytes		Whole Blood		Peripheral blood mononuclear cells		Granulocytes	
		# cells tested	Mc in gEq/10 <sup>6</sup>	# cells tested	Mc in gEq/10 <sup>6</sup>	# cells tested	Mc in gEq/10 <sup>6</sup>	# cells tested	Mc in gEq/10 <sup>6</sup>	# cells tested	Mc in gEq/10 <sup>6</sup>	# cells tested	Mc in gEq/10 <sup>6</sup>
#1	DQB1*02	204100	0	209000	0	82200	0	200200	0	209000	0	112000	0
#2	DRB1*08	200000	0	200000	0	53850	0	200000	0	200000	0	127000	0
#3	A*02	232000	0	216000	0	18000	0	219000	0	193000	0	156000	0
#4	DRB1*04	<b>165000</b>	<b>0</b>	<b>214000</b>	<b>0</b>	<b>173000</b>	<b>0</b>	<b>164000</b>	<b>0</b>	<b>227000</b>	<b>5</b>	<b>147000</b>	<b>0</b>
	DRB1*10	165000	0	214000	0	173000	0	164000	0	227000	0	147000	0
#5	DRB1*07	190000	0	219000	0	209000	0	219000	0	192000	0	216000	0
#8	DRB1*15	<b>203500</b>	<b>0</b>	<b>199100</b>	<b>66</b>	<b>134400</b>	<b>0</b>	<b>225000</b>	<b>31</b>	<b>200750</b>	<b>144</b>	-	ND
	DRB1*07	203500	0	205300	0	106000	0	200167	0	217330	0	-	ND
#9	DQB1*02	227500	0	201850	0	202800	0	205200	0	207000	0	208800	0
#10	DRB1*15	<b>209300</b>	<b>0</b>	<b>225000</b>	<b>0</b>	<b>200000</b>	<b>37</b>	<b>215800</b>	<b>88</b>	<b>202400</b>	<b>0</b>	<b>195700</b>	<b>0</b>
#11	DRB1*08	185000	0	218000	0	165000	0	256000	0	262000	0	220000	0
#12	DRB1*01	166000	0	223000	0	-	ND	159000	0	179000	0	208000	0
#13	DRB1*04	192200	0	198200	0	-	ND	196600	0	270800	0	208000	0
#14	DRB1*02	259000	0	238000	0	250000	0	212000	0	199000	0	206000	0
#15	DRB1*13	194000	0	211000	0	22600	0	193000	0	225000	0	13300	0
#17	DRB1*07	179000	0	178000	0	25900	0	252000	0	205000	0	24700	0
#18	A*03	369000	0	226000	0	101000	0	137000	0	237000	0	18400	0
#20	DRB1*03	226000	0	132000	0	40200	0	216000	0	112000	0	14480	0

Mc: microchimerism

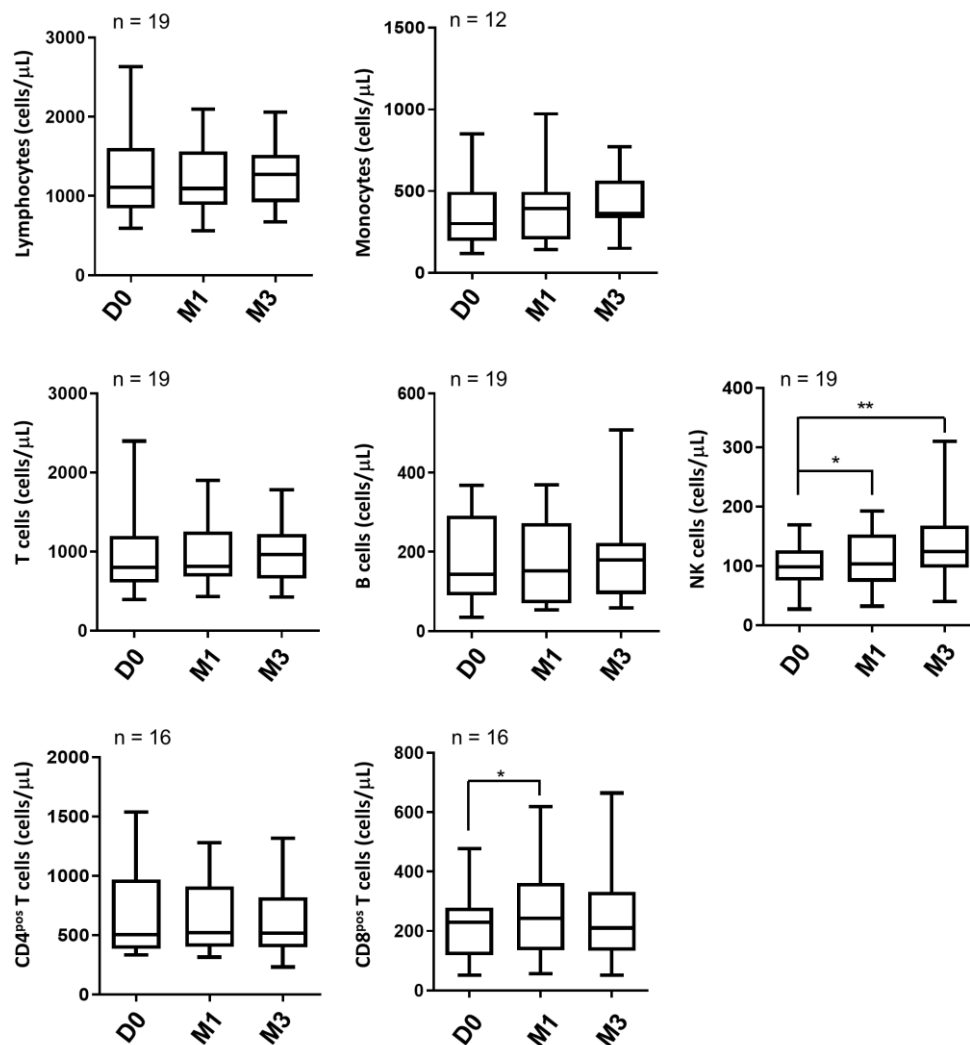
### Supplementary Table 10: Long term culture of allogenic bone-marrow mesenchymal stromal cells (BM-MSc) from products with chromosomal abnormalities

For BM-MSc final products with chromosomal abnormalities (n=3), long-term cultures were performed in accordance with the expansion protocol. Numbers of cumulative population doubling (cPD), karyotype, and hTERT expression are presented for the different cell passages. For karyotype, the number of analyzed metaphases is indicated in bracket.

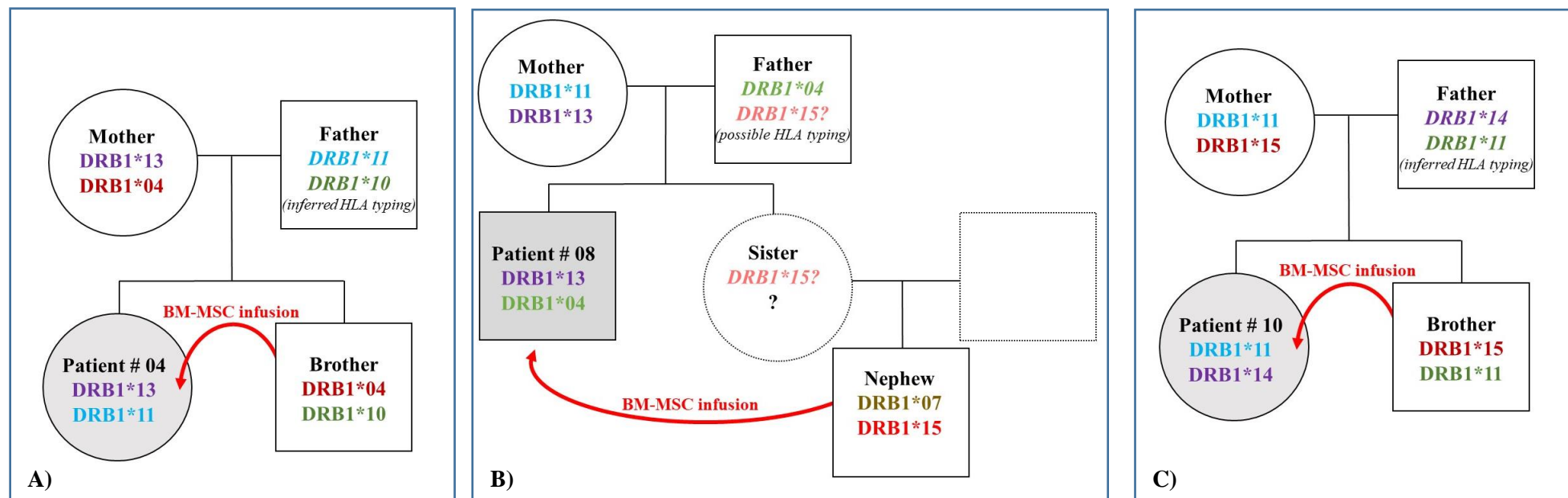
	Karyotype of final product	Long term culture			
		Passage	Number of cPD	Karyotype	<i>hTERT</i> expression
<b>Donor for Patient #3</b>	47, XX, +5 [22] ; 49, XX, +2, +5, +6 [1]; 46, XX [8]	0	-	47, XX, +5 [18] ; 46, XX [13]	No
		1	2-5	47, XX, +5 [12] ; 47, XX +2 [1]; 46, XX [17]	No
		2	6-9	47, XX, +5 [2] ; 46, XX [30]	No
		3	11-1	47, XX, +5 [5] ; 46, XX [27]	No
		4	14-9	46, XX [6]	No
		5	18-0	No metaphase	No
<b>Donor for Patient #10</b>	47, XY, +8 [2] ; 46, XY [28]	0	-	-	-
		1	1-5	46, XY [30]	No
		2	2-9	-	No
		3	8-1	46, XY [31]	No
		4	13-2	-	No
		5	17-7	46, XY [31]	-
<b>Donor for Patient #13</b>	47, XY, +5 [2]; 46, XY [28]	0	-	-	-
		1	2-3	46, XY [30]	No
		2	3-1	-	No
		3	7-1	46, XY [31]	No
		4	10-1	-	No
		5	12-1	46, XY [3]	-

## Supplementary Figure 1. Immunomonitoring of patients with severe Systemic Sclerosis after allogeneic bone marrow-mesenchymal stromal cells (BM-MSC) infusion

Circulating immune cells were quantified by flow cytometry in peripheral blood before (D0), 1 month (M1), and 3 months (M3) after MSC infusion in patients with systemic sclerosis (n= number of patients). For each population, boxplots with whiskers extended to the most extreme data point represent the repartition of cell numbers per microlitre of blood with solid line at the median value. When the Friedman test detected significant differences between means, time points were compared pairwise using Wilcoxon signed ranks test. \*:  $p \leq 0.05$ ; \*\*:  $p \leq 0.01$



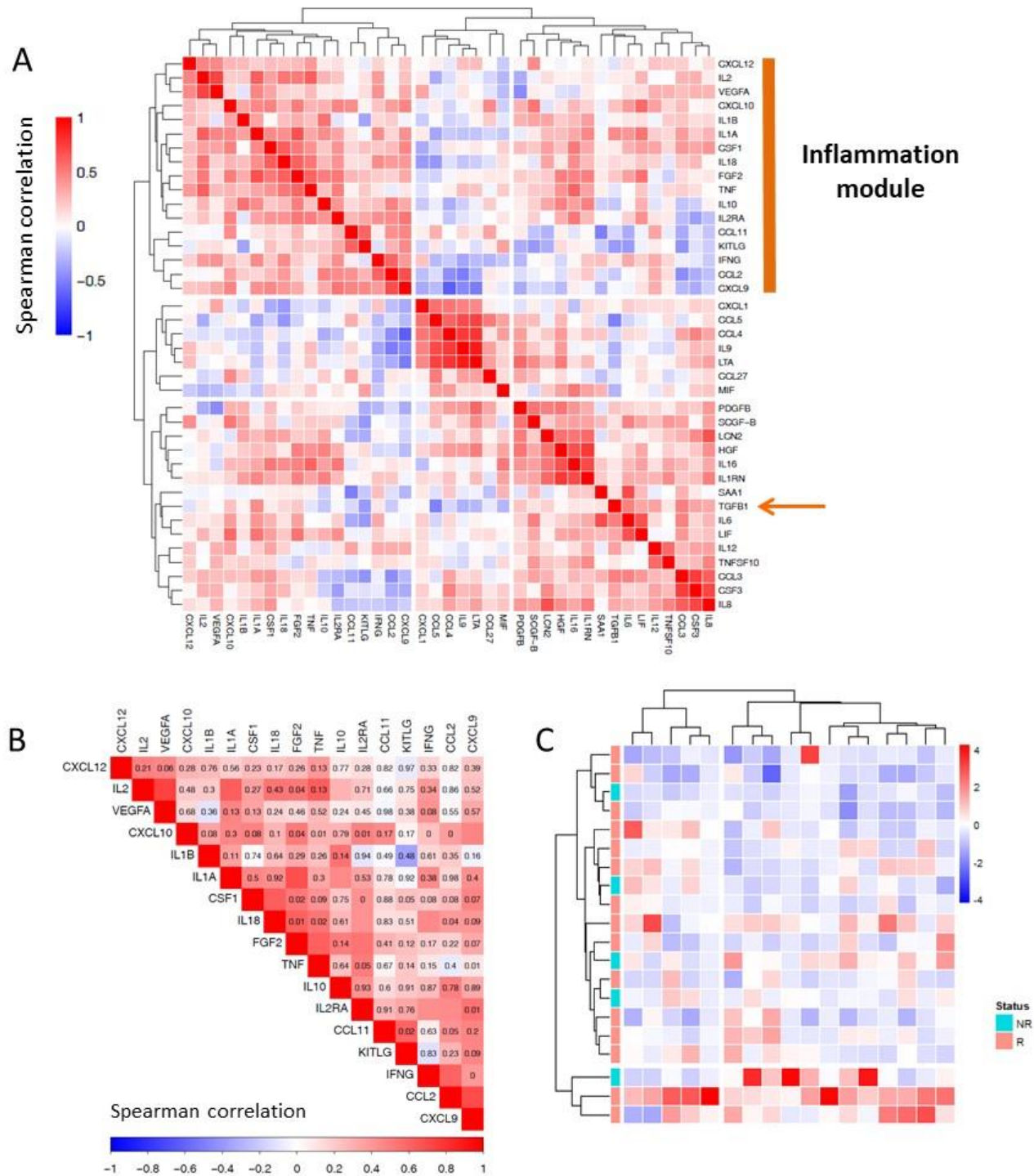
**Supplementary Figure 2. HLA typing and relationship between donor and recipient.**



(A). Patient #4 (HLA-DRB1\*11,\*13) received allogeneic BM-MSC from her HLA-mismatched younger brother (DRB1\*04,\*10). One month after MSC infusion, DRB1\*04 positive microchimerism (Mc) was detected in PBMC, while HLA-DRB1\*10 PCR remained negative in all cell subsets. In addition, mother HLA typing was DRB1\*04,\*13, indicating that the DRB1\*04 positive Mc observed in this patient was from maternal origin (B) Patient #8 (DRB1\*04,\*13) received allogeneic BM-MSC from his HLA-mismatched nephew (DRB1\*07,\*15). Only HLA-DRB1\*15 positive Mc, but not DRB1\*07, was detected prior and after MSC infusion. The patient mother was not HLA-DRB1\*15, thus, HLA-DRB1\*15 positive Mc may originate from a vanished twin carrying paternal HLA-DRB1\*15 allele, as previously shown.<sup>7</sup> The father's DNA sample was not available to confirm this point. (C) Patient #10 (DRB1\*11,\*14) whose donor HLA typing was DRB1\*11,\*15 (brother) had positive Mc for HLA-DRB1\*15, before and after BM-MSC infusion. The mother HLA typing was DRB1\*11,\*15; therefore presence of HLA-DRB1\*15-positive Mc prior to BM-MSC infusion was of maternal origin.

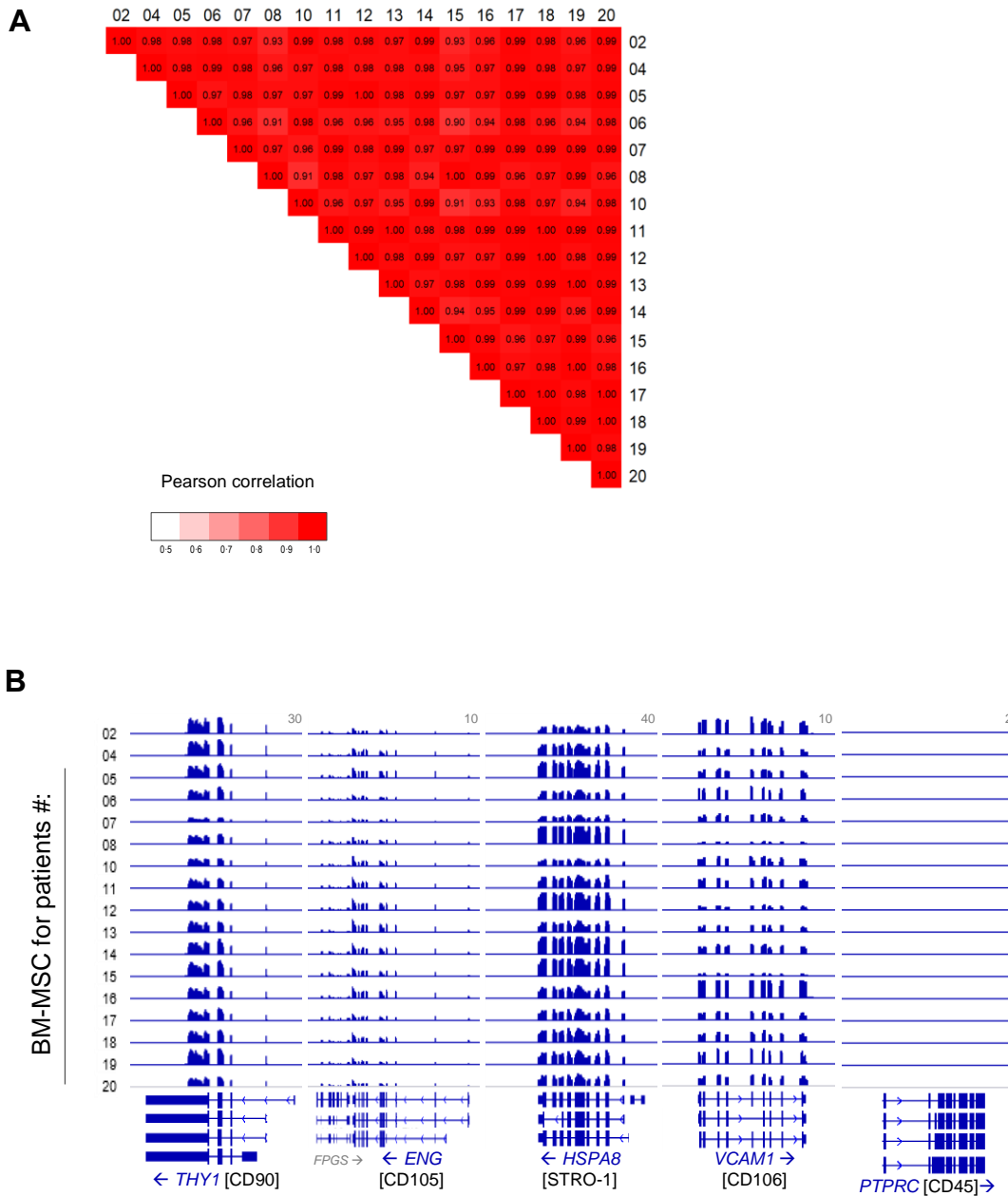
**Supplementary Figure 3. Coregulated patterns of circulating soluble factors in patients with severe Systemic Sclerosis (n=20), before allogenic bone-marrow mesenchymal stromal cells (BM-MSC) infusion.**

A) 39 parameters out of 51 were quantifiable in more than 20% of samples and represented in a Spearman correlation heatmap to evaluate all pairwise comparisons. B) Focus of the Spearman correlation heatmap on the 17 factors identified as the inflammation module (colors: correlation coefficients; values: correlation  $P$  values, all  $p$  values <0.05 are noted 0) C) Normalized values of the factors of the inflammation module are represented on a heatmap highlighting responder (R) and non responder (NR) patients.



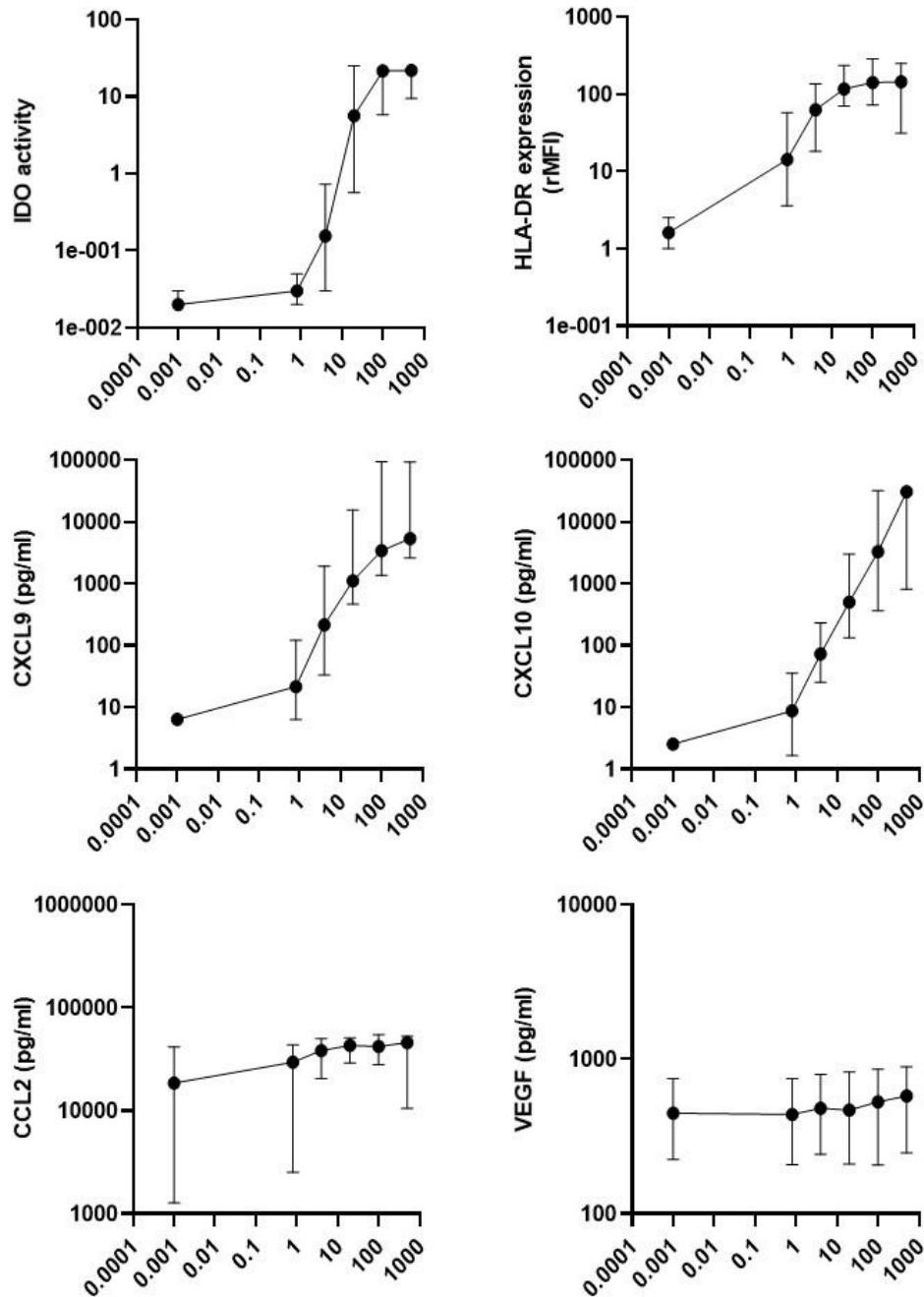
### Supplementary Figure 4. Transcriptomic analysis of the allogeneic BM-MSC final expansion products (n=17)

One aliquot of the infused P1 BM-MSC for 17 donors were analyzed at steady state by RNA-seq. A) Pearson correlation heatmap comparing the transcriptome of the P1 BM-MSC. B) Genome browser snapshots for MSC marker genes (*THY1*, *ENG*, *HSPA8* and *VCAM1*) showing the normalized RNA-seq expression profiles for each sample. All samples were negative for expression of the hematopoietic marker CD45. The gene name is indicated in blue below the gene structure, while the encoded protein is labeled in black.



**Supplemental Figure 5. Distribution of the quantifiable parameters measured upon treatment of BM-MSC batches by increasing doses of IFN- $\gamma$  (n=14).**

Each batch of BM-MSC was stimulated for 3 days with increasing doses of IFN-g before collection of culture supernatants and cells. IDO activity was quantified by mass spectrometry, soluble factors by Luminex, and HLA-DR expression by flow cytometry. Individual values of CCL2, CXCL9, CXCL10, and VEGF production, HLA-DR expression, and IDO activity are represented as median  $\pm$  range.





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