MESENCHYMAL STEM CELL THERAPY FOR CONNECTIVE TISSUE DISEASES

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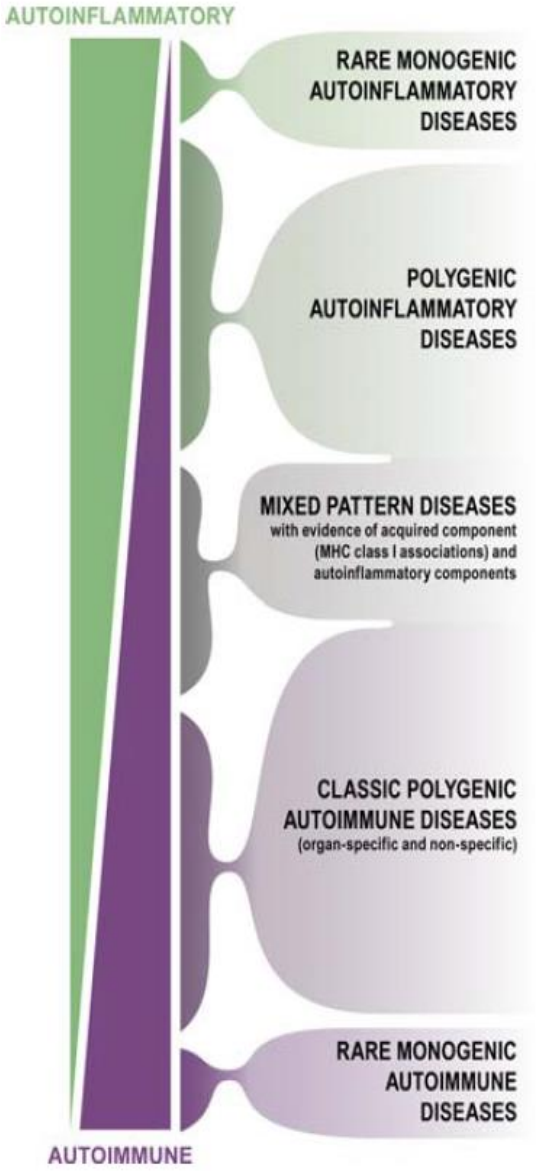
www.mathec.com

SYSTEMIC AUTOIMMUNE DISEASES: (AD):6-8 %

a single continum ≠ clinico-pathological classification



Type of Disease	Inflammatory Disorder Gene/Protein	Cellular Distribution/Function
FMF	*MEFV*/pyrin	Neutrophils, early monocyte lineage, stromal cells/regulation of inflammatory response
HIDS	*MVK*/mevalonate kinase	Widespread/cholesterol biosynthesis, prenylation
TRAPS	*TNFRSF1A*/ TNFR1	Widespread/TNF receptor
Crohn disease	*NOD2*/NOD2	Macrophages, Paneth cells/bacterial sensing
Ankylosing spondylitis Reactive arthritis Psoriasis/psoriatic arthritis Behcet Syndrome Uveitis (HLA-B27 associated)		
SLE, TID, AITD	*CTLA-4*/CTLA-4	Regulation of T lymphocytes activation
RA, SLE, T1D	*PTPN22*/PTPN22	Regulation of T lymphocytes activation
Many disorders	MHC associations	Multiple T cell functions, including B cell help
APS-1	*AIRE*/AIRE	Thymic epithelium/negative T cell selection
IPEX	*FOXP3*/FOXP3	Regulatory T cells/immunomodulation
ALPS	*FAS*/FAS	Widespread/key role in lymphocyte apoptosis

SSc : a GVHd like disease in the autologous setting!

Mc Gonagle Plus One 2006

Autoimmune + Autoinflammatory Diseases

Proposal for a Definition of Autoinflammation

Self-directed inflammation, whereby local factors at sites predisposed to disease lead to activation of innate immune cells, including macrophages and neutrophils, with resultant target tissue damage. For example, disturbed homeostasis of canonical cytokine cascades (as in the periodic fevers), aberrant bacterial sensing (as in Crohn disease), and tissue microdamage predispose one to site-specific inflammation that is independent of adaptive immune responses

Generic Definition of Autoimmunity

Self-directed inflammation, whereby aberrant dendritic cell, B and T cell, responses in primary and secondary lymphoid organs lead to breaking of tolerance, with development of immune reactivity towards native antigens. The adaptive immune response plays the predominant role in the eventual clinical expression of disease. Organ-specific autoantibodies may predate clinical disease expression by years and manifest before target organ damage is discernible.

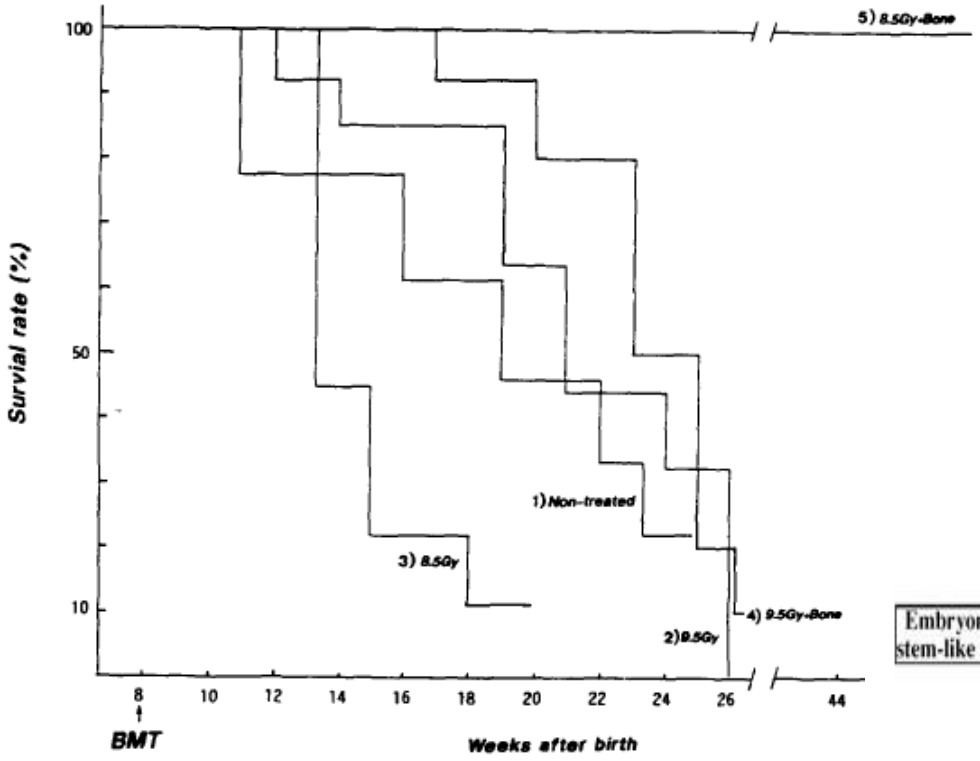
Factors determining disease manifestations	Local tissue factors at disease-prone sites, including tissue trauma, necrosis, mechanical factors, and bacteria or their constituent molecules	Clinical disease expression determined by events taking place in primary and secondary lymphoid tissues, including bone marrow, thymus, lymph nodes, and spleen
	Innate immune activation	Adaptive immune activation
Immunological basis	Genetically related to perturbations of innate immune function, including pro-inflammatory cytokine signalling abnormalities/ bacterial sensing/local tissue abnormalities	Acquired immune perturbation key-to-disease expression

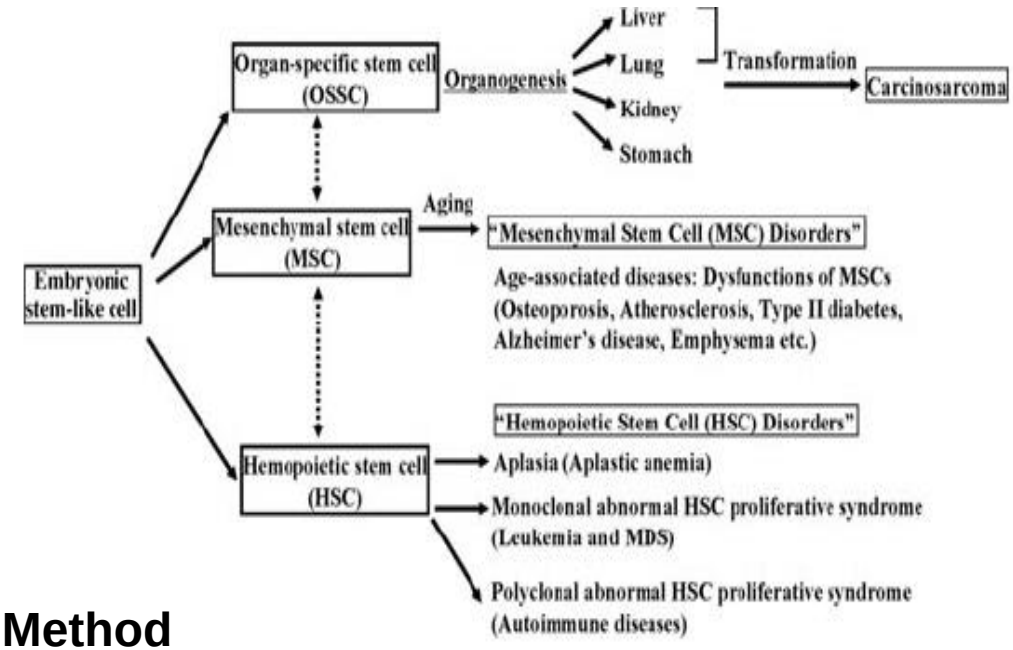
Requirement of Donor-Derived Stromal Cells in the Bone Marrow for Successful Allogeneic Bone Marrow Transplantation

Complete Prevention of Recurrence of Autoimmune Diseases in MRL/MP-lpr/lpr Mice by Transplantation of Bone Marrow Plus Bones (Stromal Cells) from the Same Donor[1]

Journal of Immunology, 1994, 152: 3119.

IKEHARA S





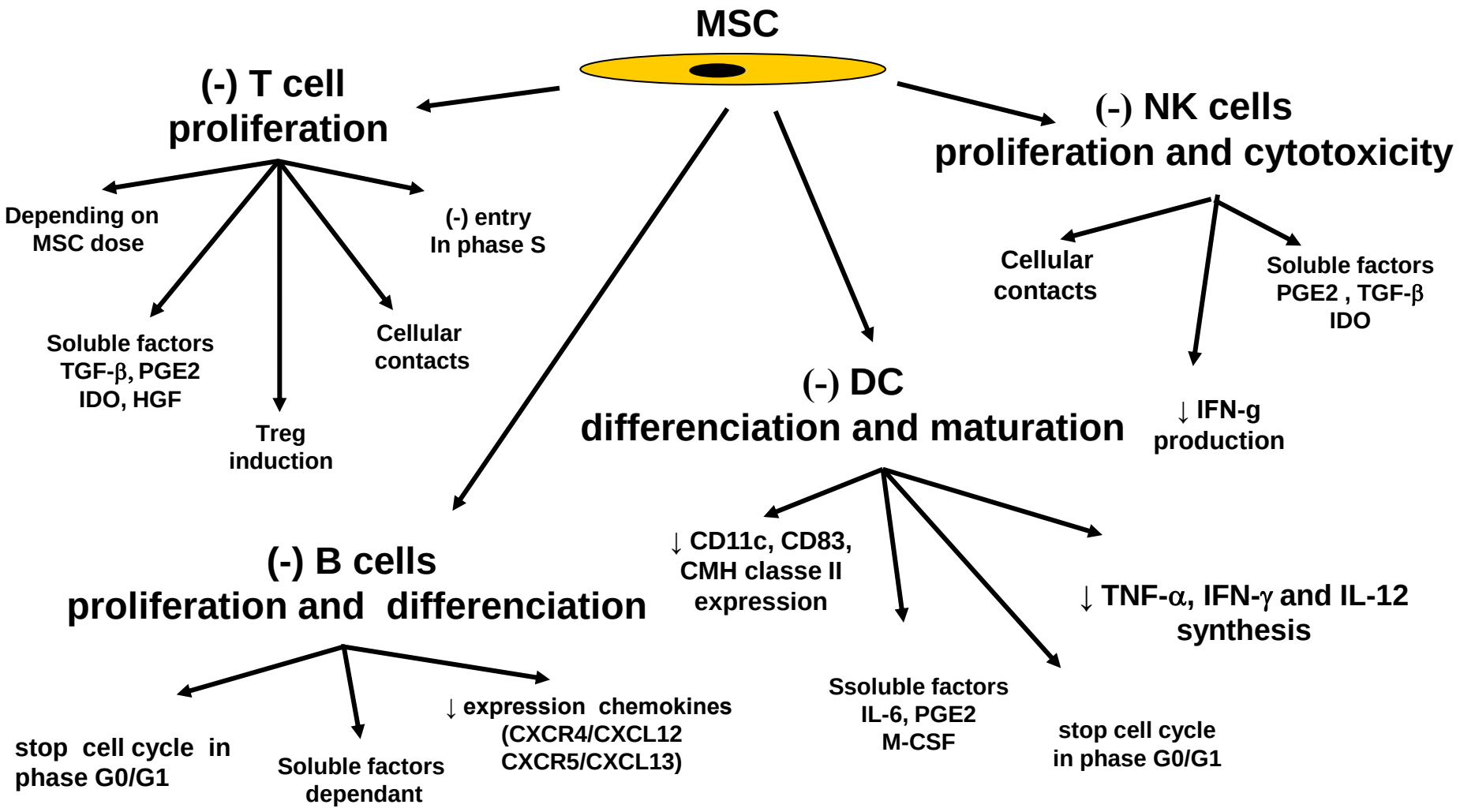
A Bone Marrow Transplantation Method for Stem cell Disorders…

Ann N Y Acad Sci 2009

Mesenchymal stem cells and immunomodulation: Toward new immunosuppressive strategies for the treatment of autoimmune diseases?

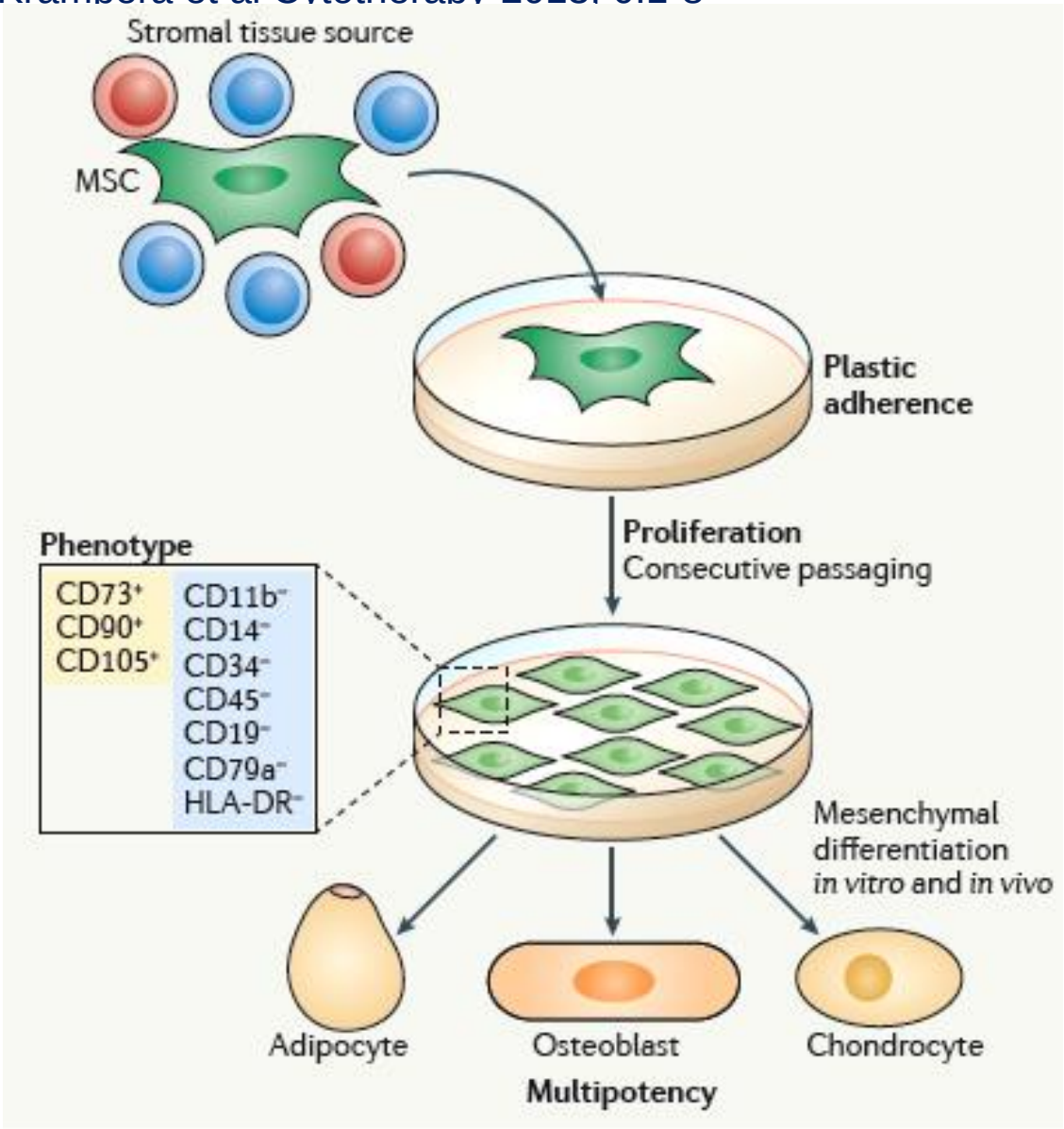
J. Larghero [a,b], L. Vija [c,d], S. Lecourt [a,b], L. Michel [c,d], F. Verrecchia [c,d], D. Farge [c,*,d]

La Revue de médecine interne 30 (2009) 287–299



Multipotent MSC: Definition and characterization

Krampera et al Cytotherapy 2013; 0:1-8



✓ Plastic adherence in standard culture conditions

✓CFU-F

✓Phenotype + ≥ 95% / - ≤ 2%

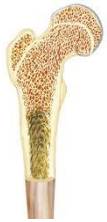
cells to exclude HSC contamination

✓ Differenciation in adipocyte,

ostéoblaste and chondrocyte

MSC sources :
BM, adipose tissue, dental tissue, umbilical cord ,

MSC : Isolation and expansion



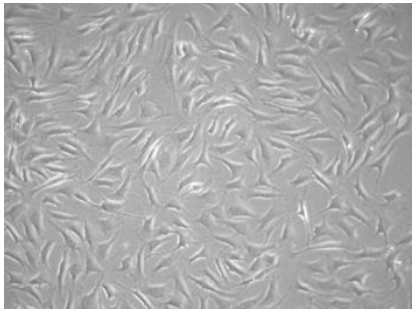
Ponction médullaire

Cellules médullaires totales

Milieu de culture
αMEM
10% SVF
FGF2

2 semaines de culture
Changement de milieu tous les 3 jours

Cellules adhérentes



Trypsinisation à confluence

Expansion

- MSC = 0.01- 0.001% Bonne Marrow mononuclear cells
- High expansion rate (25 mL BM = 2 to 5.10^8 MSC in 5 weeks)
- Limited long term proliferative potential (20-40 doubling population according age)
- Long term replication senescence

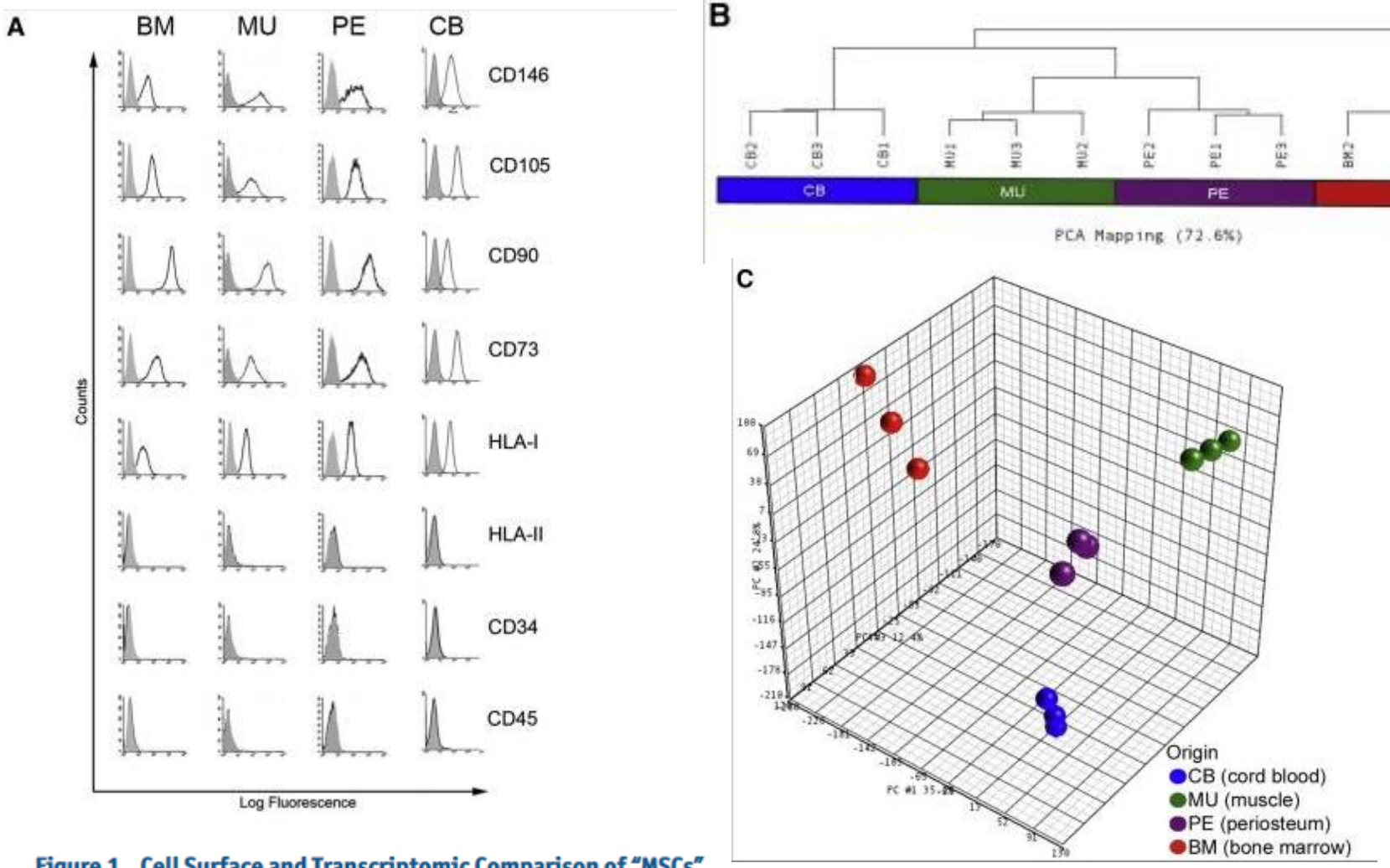


Figure 1. Cell Surface and Transcriptomic Comparison of "MSCs" from Different Tissues

(A) Fluorescence-activated cell sorting (FACS) analysis of multiclonal BM, MU (muscle), PE (periosteum) and CB cells strains express the canonical in vitro phenotype of "MSCs" and CD146 (isotype controls indicated in gray). Hierarchical clustering (B) and the principal component analysis (C) revealed that gene-expression profiles of CD146+ cells are clearly separated by the origin factor.

Sachetti, Stem cell reports, 2016

SHORT REPORT Open Access

Age, atherosclerosis and type 2 diabetes reduce human mesenchymal stromal cell-mediated T-cell suppression

Ozge Kizilay Mancini[1], Dominique Shum-Tim[2], Ursula Stochaj[3], José A. Correa[4] and Inés Colmegna[5,6*]

Kizilay Mancini et al. Stem Cell Research & Therapy (2015) 6:140
DOI 10.1186/s13287-015-0127-9

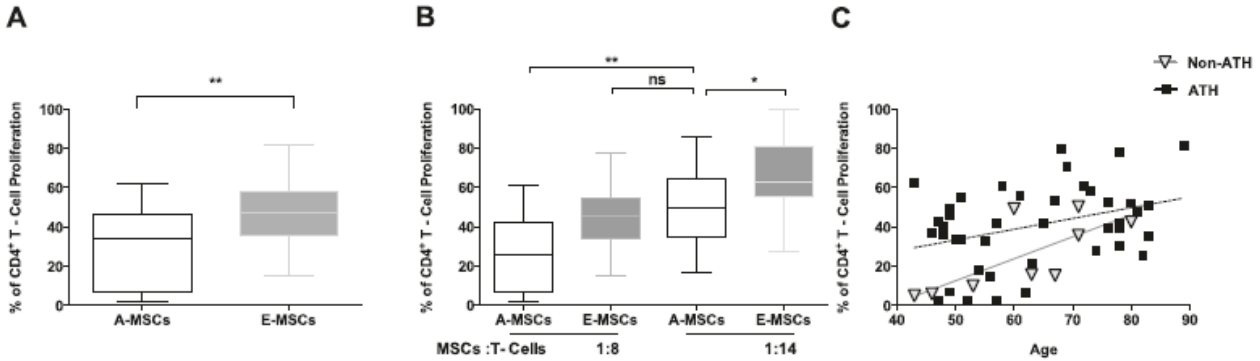


Fig. 1 Age-associated decline in mesenchymal stromal cell (MSC)-mediated $CD4^+$ T-cell suppression capacity. **a** MSCs from elderly donors (E-MSCs, ≥65 years, n = 23) are less efficient than those of non-elderly adults (A-MSCs, <65 years, n = 27) to suppress $CD4^+$ T-cell proliferation at 1:8 MSC:$CD4^+$ T cell ratio (**$p = 0.003$). **b** The suppressive effect of MSCs on $CD4^+$ T cells depends on the MSC:$CD4^+$ T-cell ratio (**$p = 0.004$). Twice the number of E-MSCs are required to affect $CD4^+$ T-cell suppression to the same extent as A-MSCs ($p > 0.9$). **c** The effect of MSC donor age on the decline of $CD4^+$ T-cell suppression is observed in patients with atherosclerosis (ATH; n = 18; $p = 0.02$, r = 0.4) and without ATH (non-ATH; n = 9; $p = 0.02$, r = 0.7)

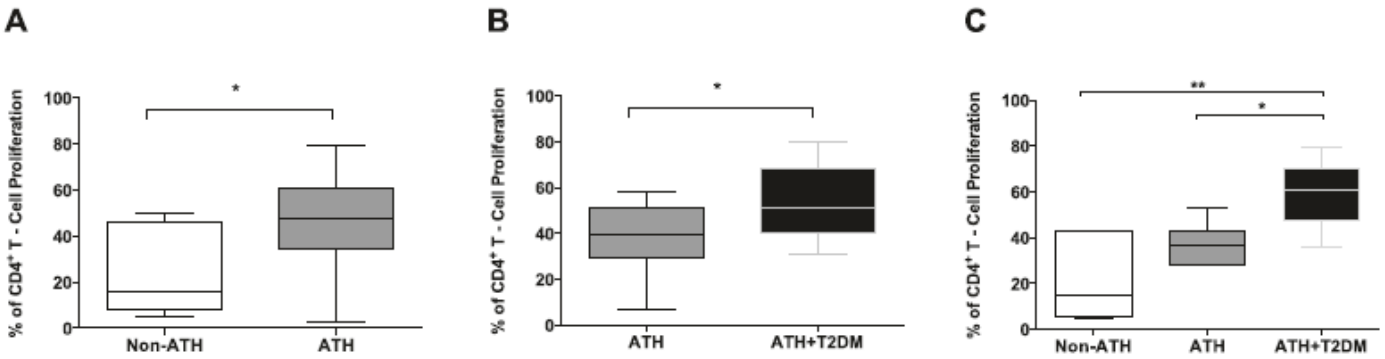
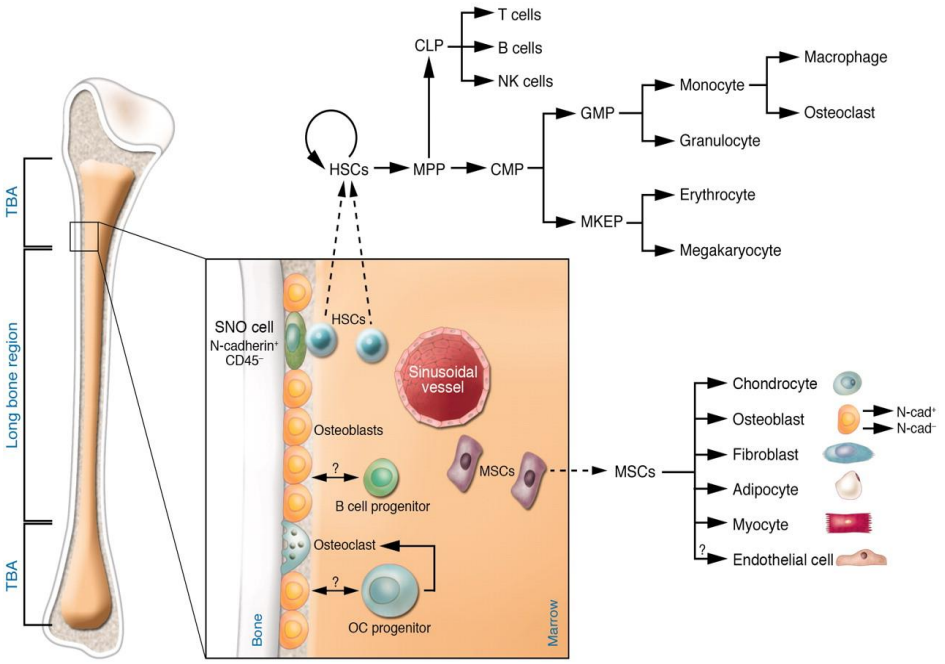


Fig. 2 Reduced MSC-mediated T-cell suppression capacity in patients with atherosclerosis and type 2 diabetes. **a** MSCs from patients with atherosclerosis (ATH; n = 18; *$p = 0.02$) have a decreased capacity to suppress $CD4^+$ T-cell proliferation at 1:8 MSC:$CD4^+$ T cell ratio compared to age-matched controls without atherosclerosis (non-ATH, n = 9). **b** MSCs from patients with ATH (n = 12) and type 2 diabetes mellitus (T2DM) (n = 12) have impaired suppressive capacity compared to age-matched ATH controls (*$p = 0.04$). **c** MSC function is compromised in age-matched patients with chronic inflammatory diseases (non-ATH < ATH < ATH+T2DM; n = 7 per group; *$p = 0.02$, **$p = 0.002$)

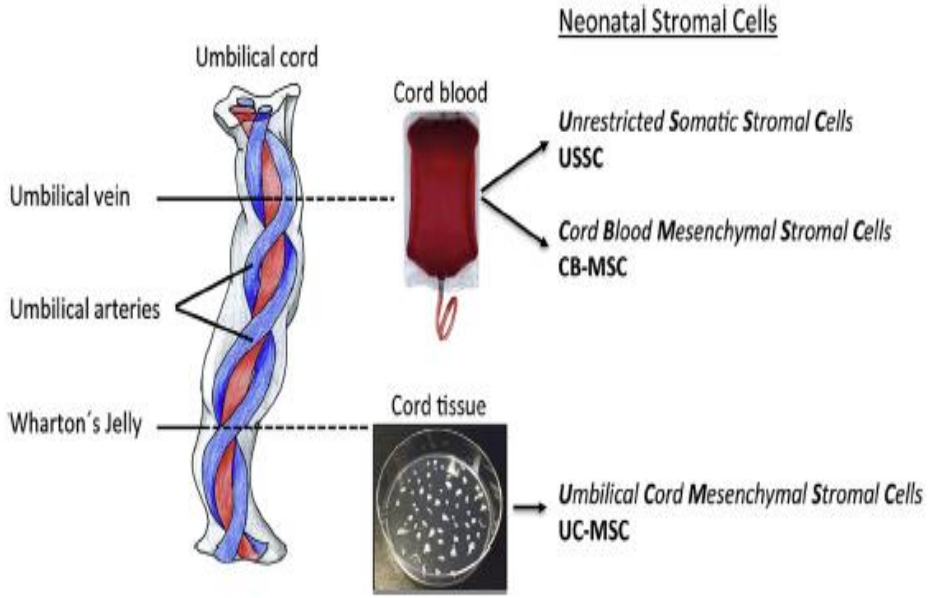
Should we use cord blood or bone marrow as a source of cells for treating AD : SC : Bone Marrow and Umbilical Cord blood human

Int J CLIN EXP Med 2010; 3: 248



MSCs = 0.001–0.01% total BM nucleated cells

- viral exposure
- proliferative/differentiation capacity with age



More primitive, less likely immunological

Allogenic use , collection easy, non invasive

no ethical / technical issues

Lower success for isolating 63% UCB vs 100% BM

Banking

1. CFUF frequency : UC > BM
2. Proliferation hUCMSC Faster (P30) > BM (P6)
3. Lower expression CD106 and HLADR UB > BM

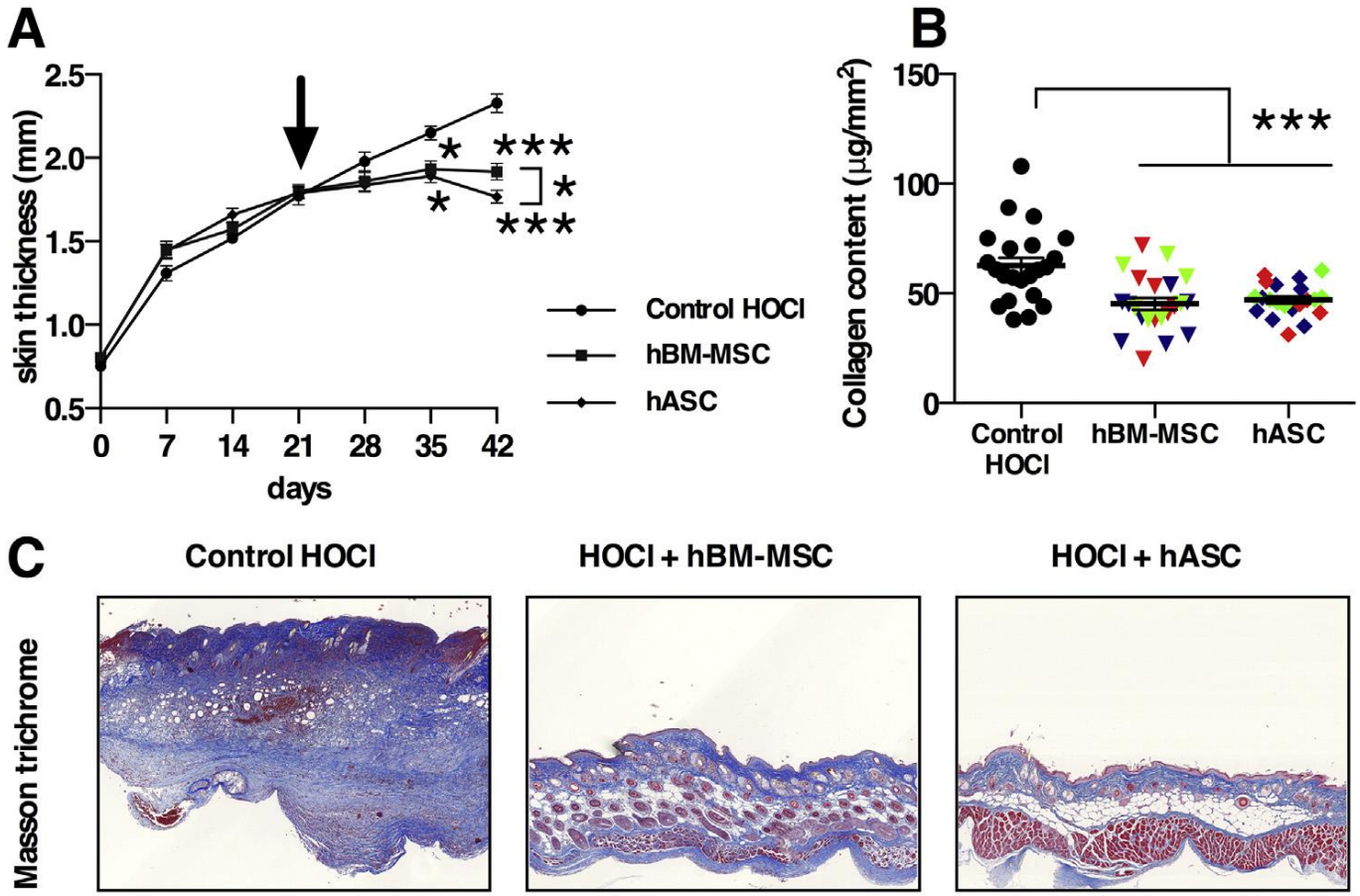


Fig. 4. Effect of hBM-MSC or hASC injection in the HOCl-induced SSc model.

(A) Skin thickness from HOCl-mice treated with an infusion of 2.5 105 hBM-MSC or hASC at d21 (arrow) compared with control HOCl-mice. 3 different samples of hBM-MSC and 3 of hASC were evaluated (each sample infused to 6e8 mice).

(B) Collagen content at d42 in skin from **control HOCl-mice**, **hBM-MSC**- **or hASC**-treated mice.

(C) Representative skin sections of mice at d42 stained with Masson trichrome, mn 10. (D) Immunostaining with DAPI (in blue) and antibodies for a-SMA (upper panels in green), CD3 (middle panels in red) or F4/80 (lower panels in green), in skin from control HOCl-mice, hBM-MSC- or hASC-treated mice, magnification 20. Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001.

Maria A, Journal of autoimmunity, 2016

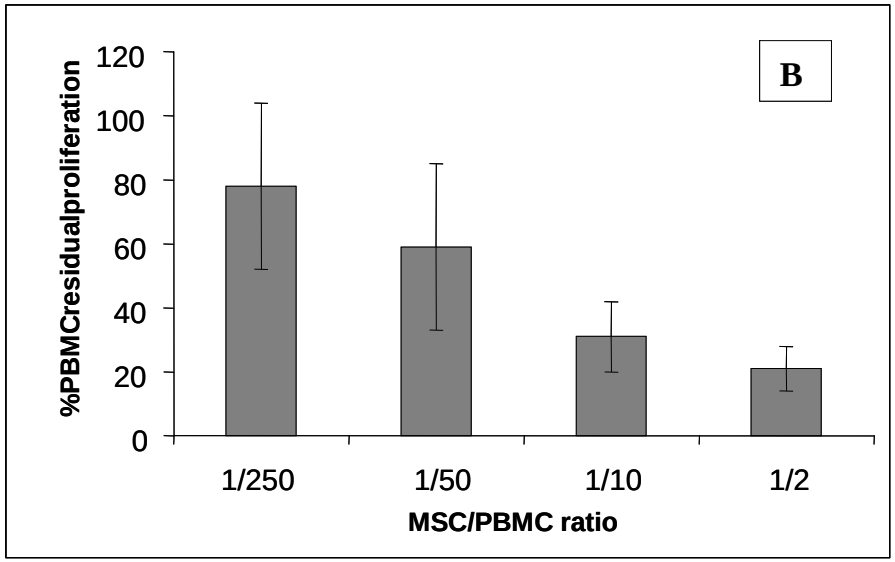
MSCs FUNCTIONS from SSc patients ? (n < 100)

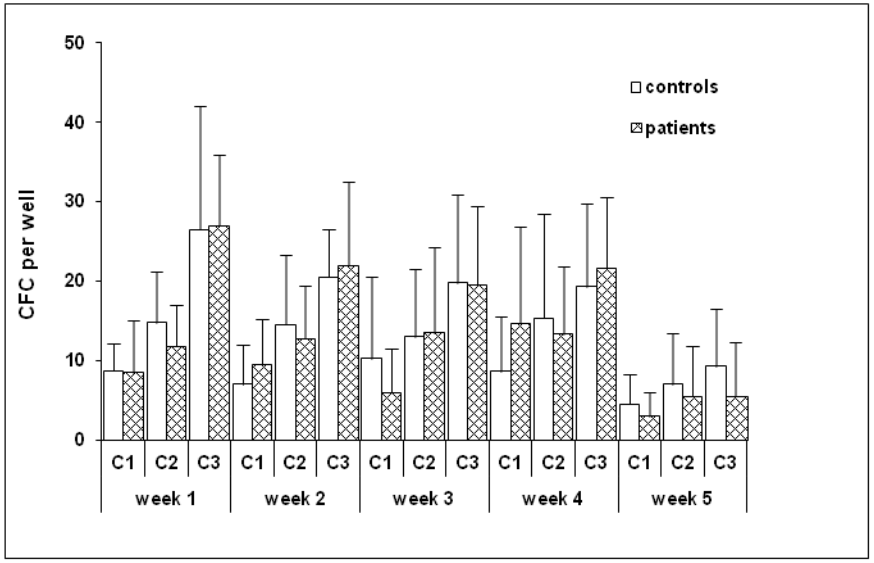
Del Papa N, Arthr Rheum 2006 (14 pts): no adipogenic /osteogenic differentiation potential, ↓ long term hematopoiesis support and early senescence.

Cipriani P Arthr Rheum 2007: ↓ in vitro endothelial differentiation but normal adipogenic/ osteogenic differentiation. Premature senescence ?

Larghero J Ann Rheum Dis 2007 Normal MSC in 12 pts + 9 C: phenotype, proliferation (CFU-F) + bFGF, differentiation, (-) CML, support hematopoiesis

Vanneaux et al *BMJ open 2012 :* ↑TGF-β RII signaling in SSc CSM



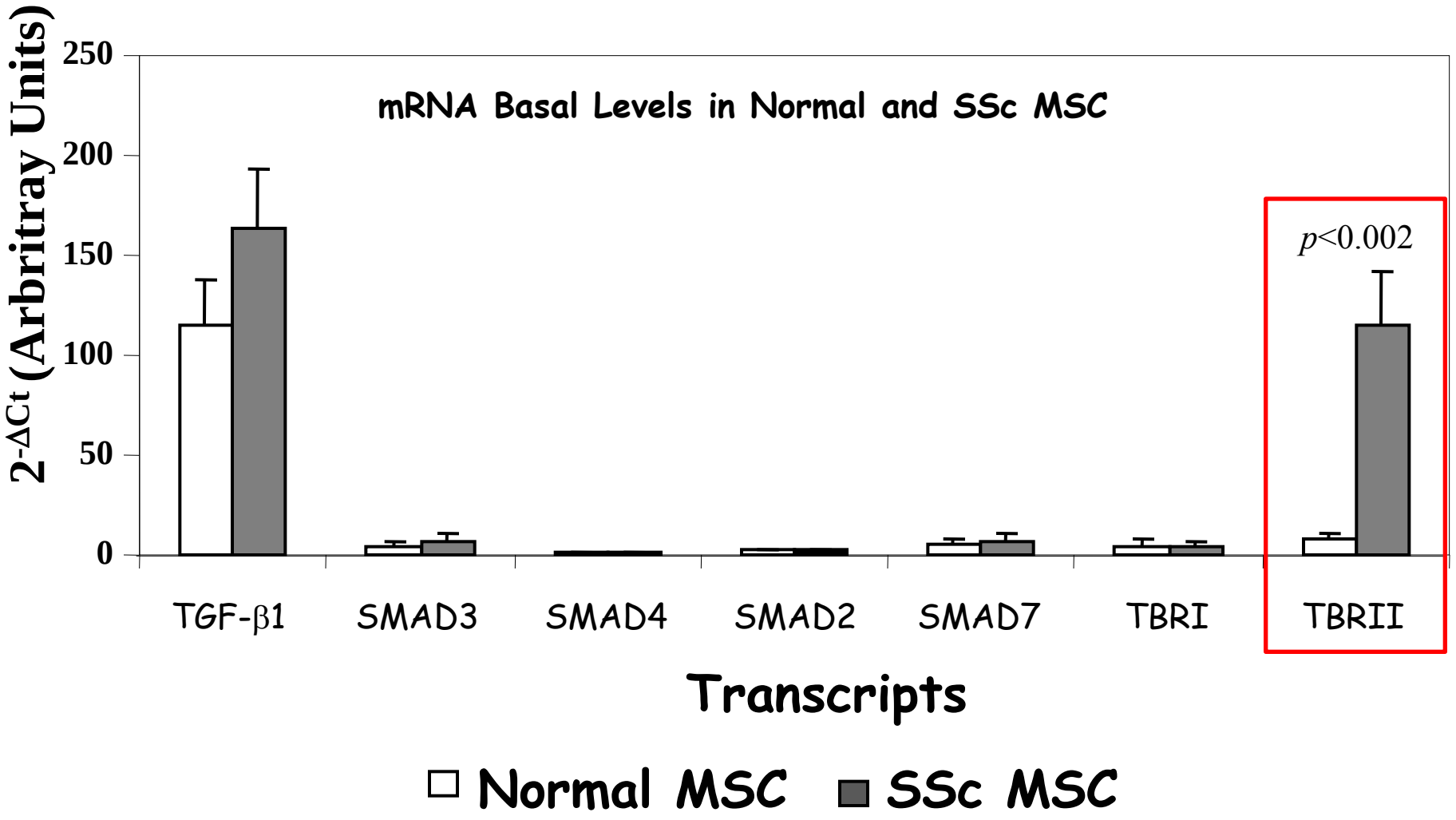


MSC IMMUNOMODULATION IN SSc PATIENTS:? since the standardisation of MSCs definition and expansion by the EBMT + International Society Cellular Therapies (ISCT)

n = 1 ***Christopeit M Leukemia 2007*** 41 yr female with advanced SSc. Allogeneic (father) BM MSC => marked improvement" 7 mth Follow Up

n= 1 Guiducci S et Al, Ann Intern Med, 2011 ♀ 24yrs, SSc with LAC+ vasculitis, resistant to Steroids, Azathioprine, Cyc, plasmaexchange. Autologous, expanded MSCs ($1x10^6$/Kg), infused at 0, +30 and + 60.

Enhanced expression mRNA levels of TGF-βRII in MSc from SSc patients



Vanneaux et al., BMJ Open 2012

Treatment of severe progressive systemic sclerosis with transplantation of mesenchymal stromal cells from allogeneic related donors – report of 5 cases

Gernot Keyßer*, M.D., Maximilian Christopeit*, M.D., Sylvia Fick*, Marit Schendel, M.D., Bettina Maria Taute, M.D., Gerhard Behre M.D., Lutz Peter Müller, M.D., Hans-Joachim Schmoll, M.D., Professor of Medicine

Arthritis & Rheumatism
DOI 10.1002/art.30431

Local anesthesia, 20ml to 120ml BM from iliac crest from donor. GMP facility. Centrifugation at 300*g, cells* resuspended With PBS, overlayed on leukocyte separation medium 1077g/l), centrifuged 30min at 300*g.*

BMMNC harvested and resuspended with growth medium. Centrifugation at 300*g*

cells were plated in growth

*Medium at 1*2.5x105BMMNC/cm2.

MSCT was carried out by intravenous infusion. Patients 1 and 2 received freshly prepared MSC, whereas patients 3-5 received cryopreserved MSC.

Disease duration/ years	Scleroderma localisation besides skin manifestation	Previous treatment	Donor of MSC/age/Date of MSCT (month-year)	Number of transplanted MSC per kg bodyweight/total number	mRSS at baseline/after 3 /after 6 months	Follow-up time (months)	Infectious adverse events (months after MSCT)
4	acral ulcerations, joints, esophagus	prednisolone, azathioprine	Father/69/9-2006	$1x10^6$ /$6x10^7$	25 /13/11	44	minor respiratory tract infection (6)
9	acral ulcerations, pericardium, pulmonary	cyclophosphamide, prostacycline infusions	Sister/41/6-2007	$0.7x10^6$ /$5.8x10^7$	23/14/17	24	minor respiratory tract infections (2;6)
2	acral ulcerations, myositis, cardiac, pulmonary	prednisolone, methotrexate cyclophosphamide	Brother /46/8-2007	$0.22x10^6$ /$1.8x10^7$	28/23/n.a.	6	minor respiratory tract infection (1)
2	myositis, pulmonary	prednisolone, methotrexate, cyclosporine	Sister/16/10-2007	$1.6x10^6$ /$10.3x10^7$	15/n.a.	4	
2	pulmonary, esophagus	prednisolone, azathioprine, cyclophosphamide	Sister/54/10-2007	$1.8x10^6$/$21.6x10^7$	23/21/23	9	minor respiratory tract infections (3;6)

Treatment of refractory severe systemic scleroderma by injection of allogenic mesenchymal stem cells

Phase I-II clinical study

Primary objective :

Feasibility and tolerance of administration of allogeneic MSCs in the treatment of severe diffuse SSc or rapidly progressive and refractory to conventional treatments by prior cyclophosphamide

Primary endpoint:

Immediate tolerance : % patients with grade III or IV adverse events (Common Terminology Criteria for Adverse Events v3.0)

Secondary objectives :

1) **Tolerance > 3 months after infusion.**
2) **Clinical response and efficacy on SSc evolution** every 3 months
3) **Immunomodulation capacity evaluation** : cytokines and autoantibodies responses

Number of patients to be included : **20**
Time of follow-up : **2 ans**
Inclusion period : **Mars/2014-Mars/2019**

Inclusion criteria

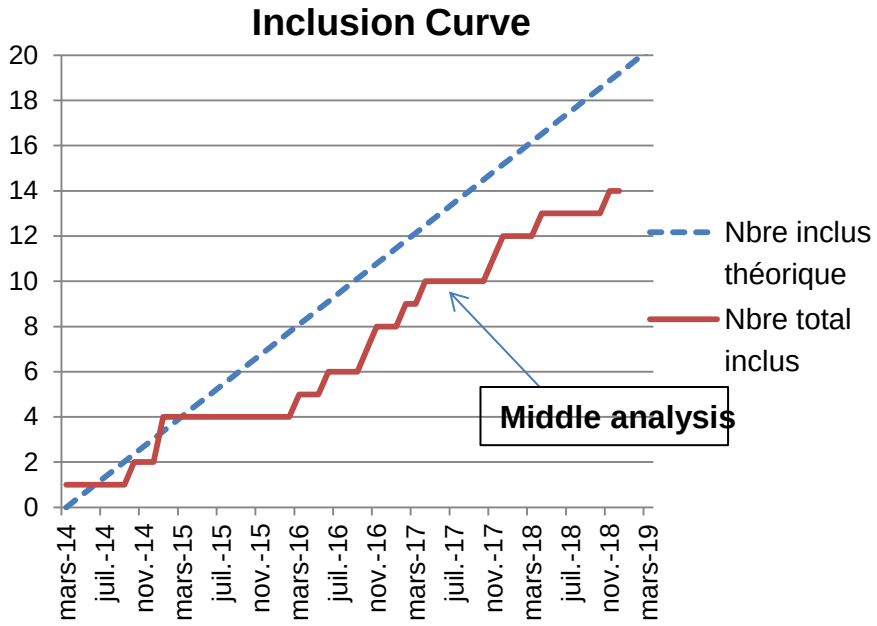
1) Age between 18 and 70
2) SSc of poor prognosis, involving life-threatening with sever visceral impairment **AND Contraindicating the use of or resistant to immunosuppressive therapy** conventionally used in severe forms of the disease according to the European recommendations of EUSTAR and EBMT
3) Severe forms with at least 6 months follow-up after completion of prior immunosuppressive therapy combined to :

- Skin lesions with **Rodnan score > 15**
- **Respiratory or Heart or Renal disease**

Treatment of refractory severe systemic scleroderma by injection of allogenic mesenchymal stem cells
Phase I-II clinical study

December 2018

14/20 patients included since 03/2014 (8 patients pts finished their 24month FU)
+ 2 patients to be included



Tolerance

- 10 premiers patients on reçu 1.10^6 MSC/kg
- 09/2017 : **middle analysis** of the primary criteria : toxicity at Day 10 after MSC

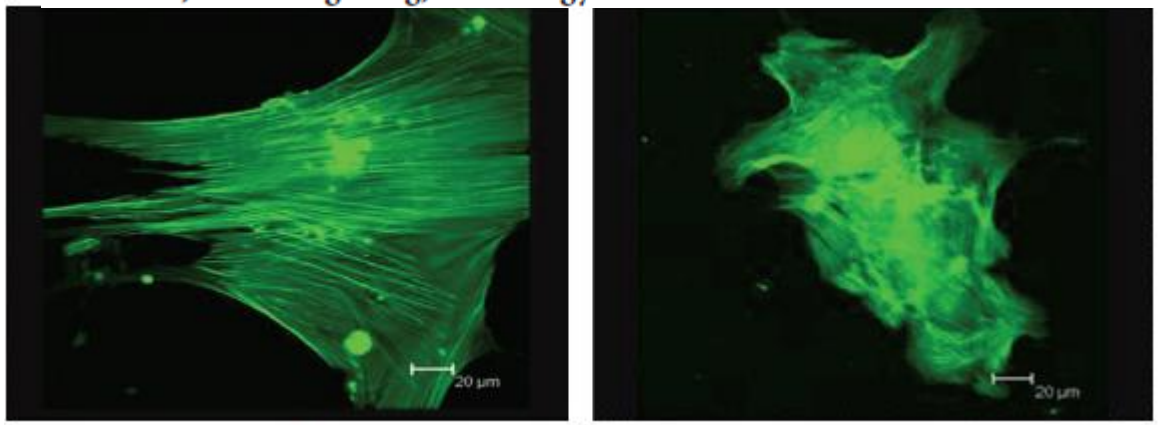
→ **increase** to **3.10^6 CSM/kg** from the 11th patient

4 patients to be included until march 2019

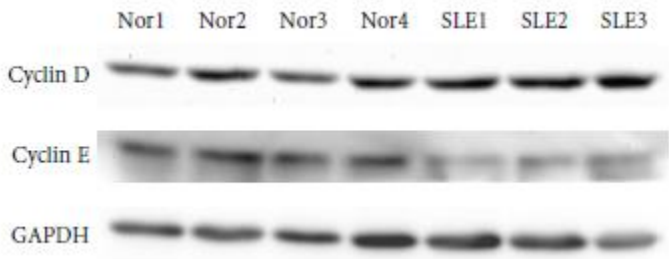
Gene Expression Profile Reveals Abnormalities of Multiple Signaling Pathways in Mesenchymal Stem Cell Derived from Patients with Systemic Lupus Erythematosus

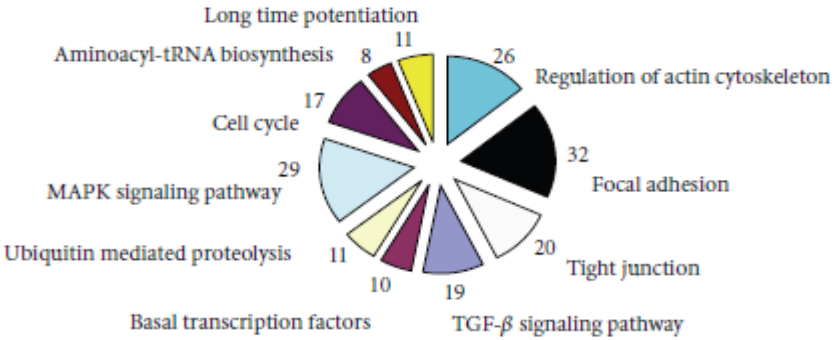
Yu Tang,[1] Xiaolei Ma,[1] Huayong Zhang,[1] Zhifeng Gu,[1] Yayi Hou,[2] Gary S. Gilkeson,[3] Liwei Lu,[4] Xiaofeng Zeng,[5] and Lingyun Sun[1]

Clinical and Developmental Immunology
Volume 2012, Article ID 826182, 12 pages



(c) (d)





Rux2, Msx2, and osterix [40]. According to the results of the microarray, most of genes in the BMP pathway were decreased including Smad-1, Smad-5, BMPR1A and the target gene Id-1. As the phosphorylation process controls the activity of Smad-1, Smad-5, and BMPR1A, we only confirmed the mRNA level of some of the target genes and the protein level of BMP-5.

patients. Furthermore, we found abnormalities in cell cycling regulation, BMP/TGF-β and MAPK pathways. Our findings suggest BMMSCs, as a component of bone marrow, may play an important role in the etiopathogenesis of SLE.

Therapeutic Effects of Umbilical Cord Blood-Derived Mesenchymal Stem Cell Transplantation in Experimental Lupus Nephritis

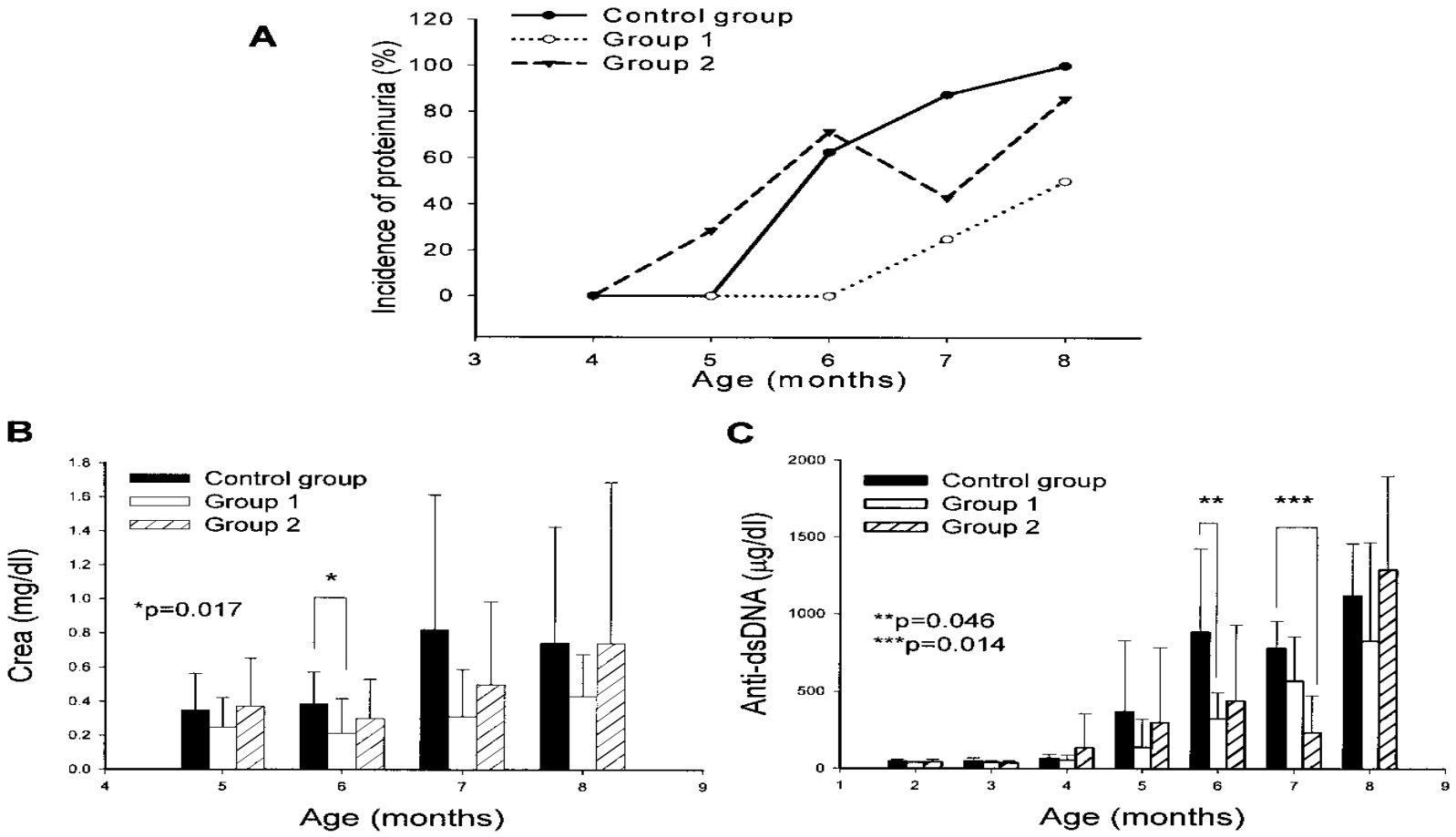
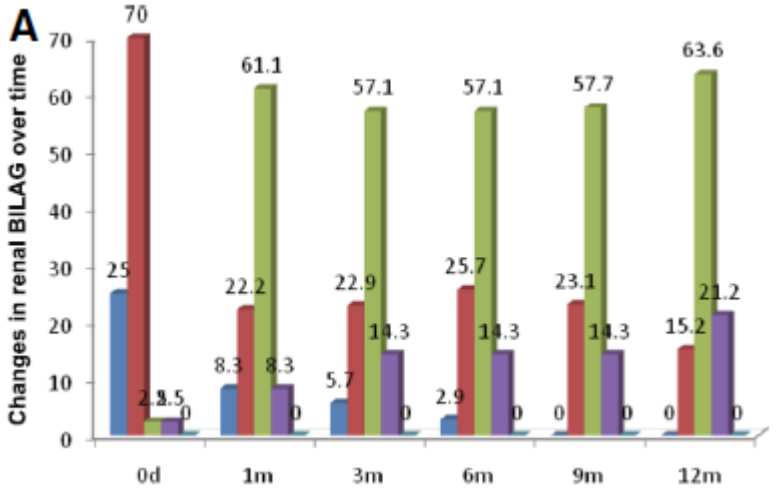
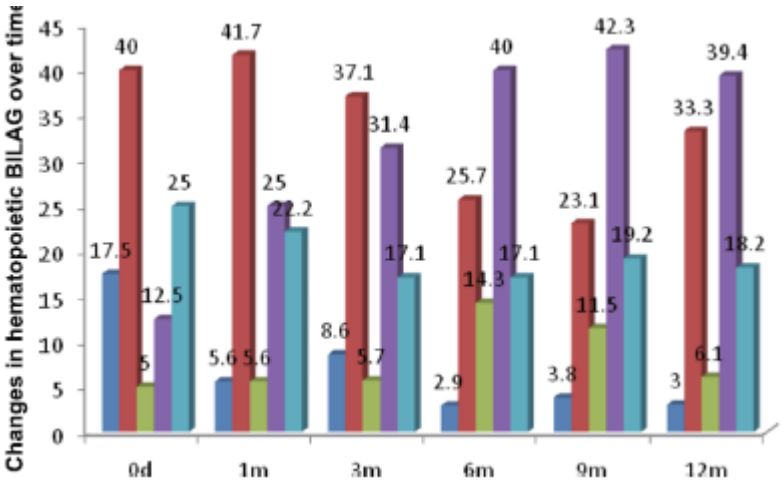


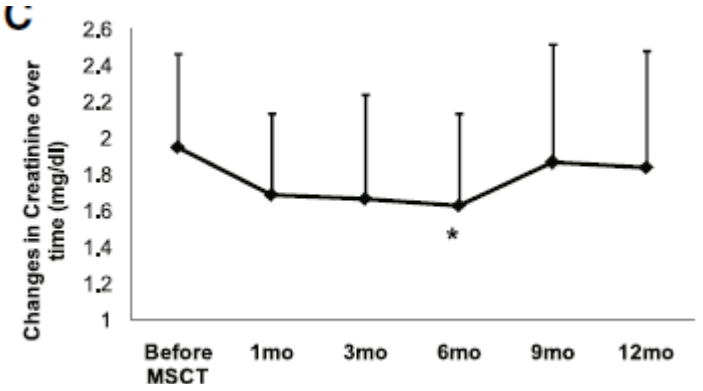
Figure 1. uMSCs improve proteinuria, serum creatinine levels, and decrease anti-dsDNA titer. (A) Incidence of proteinuria in NZB/W F1 mice. Mice treated with uMSCs transplantation showed lower incidence of proteinuria compared to the control group at 6 and 7 months of age ($p = 0.007$ and $p = 0.039$ by chi-square tests, respectively). (B) Serum creatinine levels of the experimental animals. A significant difference was found in creatinine between group 1 and the control group at 6 months of age ($*p = 0.017$). (C) Anti-dsDNA antibody titers. Group 1 showed lower levels with respect to the control group at 6 months of age ($**p = 0.046$). There was a significant difference among groups ($p = 0.019$) and group 2 showed lower levels with respect to the control group ($***p = 0.014$) at 7 months of age.

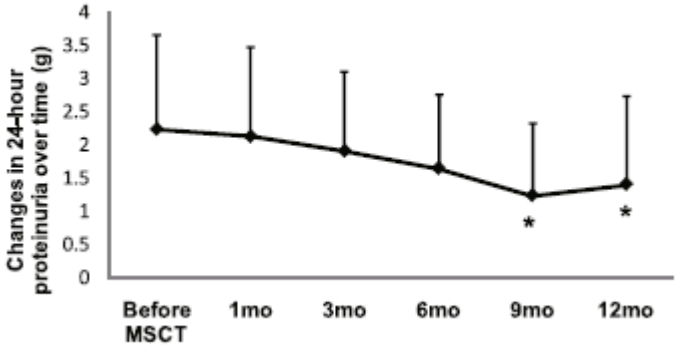
Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study

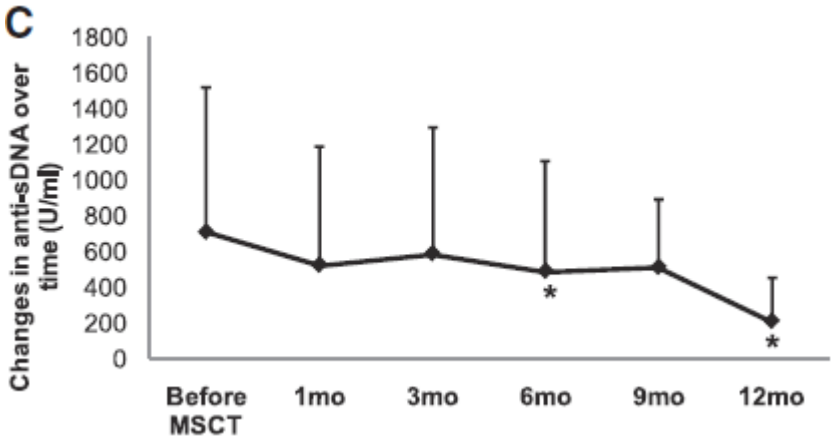
Wang *et al. Arthritis Research & Therapy* 2014, **16**:R79











Safety and feasibility of umbilical cord mesenchymal stem cells in treatment-refractory systemic lupus erythematosus nephritis: time for a double-blind placebo-controlled trial to determine efficacy

Woodword and Furst arth Resear 2016

Table 1 Overview of open-label phase I/II studies to evaluate mesenchymal stem cells (1×10^6 per kg) in treatment-refractory systemic lupus erythematosus

Authors (date)	ClinicalTrials.gov protocol number	Study design/duration of follow-up	Number of patients	MSC type/regimen	Conditioning	Safety: deaths/serious infection	PD marker[a]	Efficacy
Sun *et al.* [2]	NR	Single-arm/median of 8.25 months (range of 3 to 28 months)	16 (15 SLEN)	UC, single infusion	CYC 0.8 to 1.8 mg/kg intravenously, 2 to 4 days	0/0	Percentage of Treg cells increased at 3 months ($P = 0.03$)	'Decreasing SLEDAI and proteinuria[b] in all patients'
Liang *et al.* [3]	NCT 00698191	Single-arm/17.2 ± 9.5 months	15 SLEN	BM, single infusion	Included in protocol, but NR	0/0	Percentage of Treg cells increased at 1 week and 3 and 6 months ($P < 0.05$)	'Decreasing SLEDAI and proteinuria[b] in all patients'
Wang *et al.* [4]	NCT 00698191	Unblinded-randomized, 2-arm/12 months	58 (~88% SLEN)	BM, UC, single versus 2× (7 days apart)	CYC 10 mg/kg per day, day 4, 3, and 2	1/NR	ND	CR single: 16/30 (53%); double: 8/27 (29%)
Wang *et al.* [5]	NR	Single-arm/mean of 27 months	87 (84% SLEN)	BM, UC, single infusion, 18 patients retreated at relapse	CYC 10 mg/kg/day, day 4, 3, and 2	5/NR	ND	CR in 23/83, relapse 10/83
Wang *et al.* [1]	[c]NCT 01741857	Single-arm	40 (38 SLEN)	UC, 2× infusion, 7 days apart)	No	3/4	ND	MCR 13/PCR 11, 7 relapse

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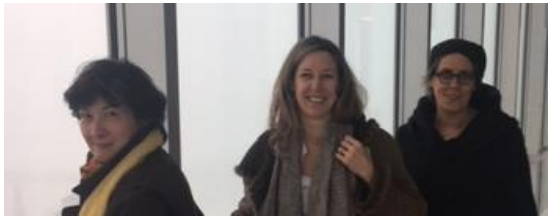

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