HSCT or MESENCHYMAL STEM CELLS for the SYSTEMIC SCLEROSIS specialist?

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**Systemic Sclerosis**

Prevalence: 7 à 1580 / M
Incidences: 0.6 à 19 / M

**FIBROSIS**
Skin, Lung digestive, heart

**HYPERVASCULAR REACTIVITY**
Raynaud, renal crisis, PHT

**AUTOIMMUNITY**
Anti Scl, Anti centromeres, Anti RNPO III

**Pathological process**
- Fibrosis
- Inflammation
- Vasculopathy

**Organ-based complication**
- Exocrine
- Pulmonary
- Cardiac
- Gastrointestinal
- Renal
- Digital ulceration
- Musculoskeletal
- Macrovacular disease

**Trends in Immunology**

Denton CP  Trends Immunol 2005
Herrick A & Cutolo M A&R 2010
Scleroderma or Systemic Sclerosis?

Prevalence: 7 à 1580 / million
Incidence: 0.6 à 19 / million x 1.2 - 1.8 black female

- **Localized Scleroderma**
  - Annular scleroderma
  - Linear scleroderma
  - Morphea
- **Systemic Sclerosis**
  - Limited SSc
  - Diffuse SSc
  - SSc sine scleroderma
  - Overlap syndrome

- No skin sclerosis proximal to elbows and knees
- Anti-centromere (ACA)
- CREST subgroup

- Proximal Skin sclerosis
- Early Inflammatory features
- Anti-Scl-70 or anti-RNA polymerase

- lcSSc or dcSSc
  + other AD features
Systemic Sclerosis: prevalence 7–500 / Million

Study or sub-category | mean difference (SE) | mean difference (random) 95% CI | Weight % | mean difference (random) 95% CI
--- | --- | --- | --- | ---
Hoyles | 4.5000 (2.5100) | | 28.67 | 4.50 [-0.42, 9.42]
Nadashkevich | 8.2000 (2.3600) | | 29.77 | 8.20 [3.57, 12.83]
Tashkin | 1.0000 (0.4500) | | 41.56 | 1.00 [0.12, 1.88]
Total (95% CI) | | | 100.00 | 4.15 [-0.51, 8.80]

Test for heterogeneity: Chi² = 10.60, df = 2 (P = 0.005), P = 81.1%
Test for overall effect: Z = 1.75 (P = 0.08)

FVC

Test for heterogeneity: Chi² = 9.93, df = 2 (P = 0.007), P = 79.9%
Test for overall effect: Z = 0.44 (P = 0.66)

DLCO

Nanini Athr Res Ther 2008
Time-dependent hazard, P=0.01

<table>
<thead>
<tr>
<th>FU (yr)</th>
<th>HR (95%CI), P-value</th>
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<tbody>
<tr>
<td>1/4</td>
<td>2.45 (.76 - 7.89), 0.13</td>
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<tr>
<td>1/2</td>
<td>1.42 (.58 - 3.51), 0.44</td>
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<tr>
<td>1</td>
<td>0.39 (.18 - .82), 0.014</td>
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<tr>
<td>2</td>
<td>0.22 (.08 - .58), 0.002</td>
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<tr>
<td>8</td>
<td>0.22 (.08 - .58), 0.002</td>
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ASTIS trial March 2013
JvL*–D*F–AT (May 2012 data cut-off)
CLASSIFICATION: AUTOIMMUNE DISEASES (AD): 6-7%

Clinico-pathology

**Systemic AD**: LED, Sjogren, Scleroderma, RA
Dermatomyositis, Polymyositis

**Organ / tissue specific**

**Endocrinology**: TID, Hashimoto’s, Thyroiditis
Addison

**Gastro enterology**: Coeliac Diseases, Crohn,
Dermatology: Pemphigus vulgaris, Vitiligo

**Haematology**: HA, TIP

**Neurology**: Myasthenia

Physiopthaological Continuum

Mc Gonagle Plus One 2006
 MSC in AID: WHICH RATIONALE?
Growth factors and cytokines synthesis

**Sources:**
Bone marrow, Fat, Umbilical cord, Placental membranes, Amniotic fluid epithelial Synovial membrane

**Self renewal + differentiation capacities**

**Regenerative medicine:** repair of damaged tissue
**Immunomodulation:** homing to inflammed tissue then antiinflammatory effects
A new BM Transplantation Method for Stem cell Disorders...a new concept for AD PERFUSION METHOD + INTRA BONE IKEHARA S Ann N Y Acad Sci 2009
Mesenchymal stem cells and immunomodulation: Toward new immunosuppressive strategies for the treatment of autoimmune diseases?

- **T cell proliferation**: Depending on MSC dose.
  - Soluble factors: TGF-β, PGE2, IDO, HGF.
  - Cellular contacts.
  - Treg induction.
- **B cell proliferation and differentiation**: Stop cell cycle in phase G0/G1.
  - Soluble factors dependant.
  - ↓ expression chemokines (CXCR4/CXCL12, CXCR5/CXCL13).
- **NK cells proliferation and cytotoxicity**: Cellular contacts.
  - Soluble factors: PGE2, TGF-β, IDO.
  - ↓ IFN-g production.
- **DC differentiation and maturation**: ↓ CD11c, CD83, CMH classe II expression.
  - Soluble factors: IL-6, PGE2, M-CSF.
  - Stop cell cycle in phase G0/G1.
  - ↓ TNF-α, IFN-γ and IL-12 synthesis.
MSCs in vitro: major immunomodulatory properties support their use for AID

- **Low immunogenicity:**
  intermediate levels of MHC class I => safe transplantation across major HLA complex barriers. Le Blanc K, Exp Haematol 2003

- **Effects on T cells:**
  (-) CD4 / CD8 / memory / naïve T cell proliferation Di Nicola M Blood 2002  
  (+) CD8+Treg production (- lymphocyte proliferation in allogeneic transplantation) Maccario R. Haematologica 2005

- **Effects on B cells:** (-) B cell proliferation and activation in a dose dependent manner Corcione et al. Blood 2006

- **Effects on dendritic cells:** (-) DC differentiation mediated by IL-6; M-CSF, PGE2 Nauta et al. J Immunol 2006; Djouad F et al. Stem Cells 2007

- **Effects on NK cells:** (-) NK proliferation mediated by IL-2 or IL-15, and the IFNg production Sotiropolou PA Stem Cells 2006
**In-vivo Models Tissue injury: MSC Homing**

Rat kidney ischaemia/reperfusion RFailure  
*Togel F Kidney Int* 2005  
Renal failure/fibrosis COL4A3 deficient mouse Ninichuck *Kidney Inter* 2006  
- localised to kidney, no transdifferentiation, reduced fibrosis  
- ↑ vasculature (VGEF, BMP-7) + NO reduction in renal failure

Mouse bleomycin lung fibrosis *Ortiz L Proc Natl Acad Sci USA* 2003  
- home to affected lung, ↓ inflammation and fibrosis  
- most effective immediately after injury

Carbon tetrachloride hepatic fibrosis mouse *Fang B Transplantation* 2004  
- protect immediately, not one week later  
- some transdifferentiation to epithelium

- reduced necrosis and infiltration “leukocyte diversion”
MSc Mechanism of immunomodulation and homing  
Yahgi H et al
Cell transplantation 2010

Figure 1. Mechanism of antigen presentation and immunomodulation
At low levels of IFN-γ, MSC express MHC-II as APCs and at high levels of IFN-γ, MHC-II is downregulated and B7-H1 is upregulated. IFN-γ, and TNF-α individually stimulate MSCs to upregulate PGE2, COX-2 and/or IDO. These mediators can inhibit T-cell, NK-cell, and DC function. MSC expresses TNFR and TLR which regulates NFkB activation. This pathway modulates the cytokine secretion from MSCs and the inhibition of T-cell proliferation.

Figure 2. Mechanism of homing
A) MSCs express integrin β1 and/or integrin α4/β1 complex stimulated by cytokines such as TNFα and IL-1. They home to VCAM-1 expressed endothelial cells that are primed by local inflammation. B) MSCs can express CXCR4 stimulate by tissue injury and modulate cell-cell contact and rolling with endothelial cells that upregulate SDF-1 due to tissue injury, such as hypoxia. C) Finally, MSCs transmigrate into extracellular matrix by interactions with integrins and fibronectin which is modulated by bFGF, TLR signaling, or MMP-2 expressed by MSCs.
Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

Katarina Le Blanc, Ida Rasmusson, Berit Sundberg, Cecilia Götherström, Moustapha Hassan, Mehmet Uzunel, Olle Ringdén

Adult bone-marrow-derived mesenchymal stem cells are immunosuppressive and prolong the rejection of mismatched skin grafts in animals. We transplanted haploidentical mesenchymal stem cells in a patient with severe treatment-resistant grade IV acute graft-versus-host disease of the gut and liver. Clinical response was striking. The patient is now well after 1 year. We postulate that mesenchymal stem cells have a potent immunosuppressive effect in vivo.

Lancet 2004; 363: 1439–41
See Commentary page 1411

A 9-year-old boy with acute lymphoblastic leukaemia in third remission received a transplant of blood stem cells from an HLA-A, HLA-B, HLA-DRβ1 identical, unrelated, female donor after conditioning with cyclophosphamide (120 mg/kg) and fractionated total body irradiation (3 Gy for 4 days). Immunosuppression included thymoglobulin (6 mg/kg) during the conditioning, followed by ciclosporin combined with four doses of methotrexate. On day 11 after allogeneic stem-cell transplantation, the patient developed a maculopapular rash of the thorax and back that progressed despite treatment with prednisolone (2 mg/kg daily). By

Female epithelial cells in the colon detected by X chromosome and Y chromosome FISHA: Cells stained with 4′6-diamidino-2-phenylindole-2HCl nuclear counterstain in 3-μm-thick slides (×100). B: Cells double-stained for cytokeratin (×20). C: Cells stained for CD68 to exclude macrophage contamination (×20). Arrows indicate XX-positive cells.
Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study

Katarina Le Blanc, Francesco Frassoni*, Lynne Ball*, Franco Locatelli, Helene Roelofs, Ian Lewis, Edoardo Lanino, Berit Sundberg, Maria Ester Bernarda, Mats Remenber, Giorgio Dini, R Mearten Egeler, Andrea Bacigalupo, Willem Fibe, Olle Ringdén, on behalf of the Developmental Committee of the European Group for Blood and Marrow Transplantation

Summary

Background Severe graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic transplantation with haemopoietic stem cells. Mesenchymal stem cells modulate immune responses in vitro and in vivo. We aimed to assess whether mesenchymal stem cells could ameliorate GVHD after haemopoietic-stem-cell transplantation.

Methods Patients with steroid-resistant, severe, acute GVHD were treated with mesenchymal stem cells, derived with the European Group for Blood and Marrow Transplantation ex-vivo expansion procedure, in a multicentre, phase II experimental study. We recorded response, transplantation-related deaths, and other adverse events for up to 60 months' follow-up from infusion of the cells.

Findings Between October, 2001, and January, 2007, 55 patients were treated. The median dose of bone-marrow derived mesenchymal stem cells was 1.4x10^6 (min-max range 0.4-9x10^6) cells per kg bodyweight. 27 patients received one dose, 22 received two doses, and six three to five doses of cells obtained from HLA-identical sibling donors (n=5), haploidentical donors (n=18), and third-party HLA-mismatched donors (n=6). 90 patients had a complete response and nine showed improvement. No patients had side-effects during or immediately after infusions of mesenchymal stem cells. Response rate was not related to donor HLA-match. Three patients had recurrent malignant disease and one developed de-novo acute myeloid leukaemia of recipient origin. Complete responders had lower transplantation-related mortality 1 year after infusion than did patients with partial or no response (11 [37%] of 30 vs 18 [72%] of 25; p=0.002) and higher overall survival 2 years after haemopoietic-stem-cell transplantation (16 [53%] of 30 vs four [16%] of 25; p=0.018).

Interpretation Infusion of mesenchymal stem cells expanded in vitro, irrespective of the donor, might be an effective therapy for patients with steroid-resistant, acute GVHD.

MSCs FUNCTIONS from SSc patients? (n < 40)

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
TGF-β signaling

1. Liaison du TGF-β à son récepteur TβRII
2. TβRII recrute TβRI et activation de TβRI
3. Phosphorylation et activation de Smad 3

Voies indépendantes de Smad
- p38
- JNK
- PI3K
- ERK

Voie Smad
- Smad 2/3
- Smad 4
- Smad 7

Smad 2/3 phosphorylation et activation de TβRI

Formation d’hétérocomplexes

Translocation dans le noyau

Régulation de l’expression génique

Cibles de Smad3: COL1A1, COL1A2, COL3A1 et COL5A2
Enhanced expression of TGF-βRII transcript in MSc Scleroderma patients

Vanneaux et al., BMJ Open 2012
Expression COL1α2 fibroblasts

Vanneaux et al., BMJ Open 2012

n = 9 SSc vs n = 9 Controls

Smad activation (WB)
Marked improvement of severe progressive SSc after TP of MSC from an allogeneic haploidentical-related donor mediated by ligation of CD137L

Christopeit M Leukemia, 1Nov 2007

- 41 yr old F patient, 4 yr duration SSc
- **Before MSC:** mRSS: 25, skin ulceration, acral sclerosis, 7.5 mg pred + 100mg AZA/d
- Allogeneic IV$10^6$ MSCs /kg BW
- **After MSCs:** 7 mths: 5/6 skin ulcerations recovery
  1 yr: mRSS 11, VAS 2 (vs5), no organ dysfunction

\[ \text{\textit{N}} = 1 \] Guiducci S et Al, Ann Intern Med, 2011

♀ 24yrs, SSc with LAC+ vasculitis, resistant to Steroids, Azathioprine, Cyc, plasmaexchange. Autologous, expanded MSCs (1x$10^6$/Kg), infused at 0, +30 and + 60.
Local anesthesia, 20ml to 120ml BM from iliac crest from donor. GMP facility. Centrifugation at 300g, cells resuspended with PBS, overlayed on leukocyte separation medium 1077g/l), centrifuged 30min at 300g. BMMNC harvested and resuspended with growth medium. Centrifugation at 300g cells were plated in growth Medium at 12.5x105BMMNC/cm2. MSCT was carried out by intravenous infusion. Patients 1 and 2 received freshly prepared MSC, whereas patients 3-5 received cryopreserved MSC.
Toxicity of MSCs

- Mouse melanoma metastasis model enhanced by prior MSCs: Local infiltration and distant effect *Djouad F Blood, 2003*

- Accumulated Chromosomal instability in murine BM MSC leads to malignant transformation *Miuria M Stem Cells 2007*

- Mouse sarcoma – ex vivo expanded, luciferase labelled and unlabelled MSCs induced sarcomas (lungs and extemities) in irradiated allogeneic recipients *Tolar J Stem Cells 2007*
Primary objective: Feasibility and tolerance of allogeneic MSC treatment for severe SSC refractory to Cyclo iv or AHSC

Primary Outcome: Immediate tolerance % pts with at least one grade III or IV secondary effects according to CTCAE (Common Terminology Criteria for Adverse Events v3.0)

Secondary Outcome:
1) Tolerance: 3 mths after injection (no malignancy)
2) Clinical response SSc: evolution on a quaterly FU up to 2 yrs
3) Immune reconstitution and immunomodulation

Healthy allogeneic donor intrafamilial.

Dose of injected MSC: 1 x 10^6 CSM /kg body wt of the recipient

Patient selection: severe progressive SSC resistant to CY or HSCT

Patient number: 20 patients total in 3 years
PHRC 2011 Phase I –II allogeneic MSSc in SSc
D Farge, PHRC 2011
St Louis Hospital, AP-HP INSERM U 976,

- **Patient number**: 20 patients total in 3 years
- **Statistical analysis**
  - First 10 patients initial dose 1 $10^6$ CSM par kg weight
  - 10 following patients:
    1) - 0.5 $10^6$ CSM / kg if high probability of excess toxicity at 1 $10^6$ CSM par kg
    2) - 3 $10^6$ CSM / kg if low probability of excess toxicity at 1 $10^6$ CSM par kg
    3) - 1 $10^6$ CSM par kg if no of the above.
- **Early cessation of the trial at the minimal dose if**
  - 1) any death or vital complications up to 3 months after injection related to treatment in 2/4 patients (stop if > 2/4)
  - 2) neutropenia < 500 /mm3 at D 30 after reinjection in 2/4 patients (stop if > 2/4)
  - 3) if SSc progression in 2/4 patients (stop if > 2/4)
HOW TO DESIGN THE NEXT TRIAL

1 CLINICAL TRIAL DESIGN

2 ACCREDITATION FOR PRODUCTION

3 ACCREDITATION FOR INJECTION

4 GOOD CLINICAL PRACTICE For CLINICAL TRIAL
PHRC funded in April 2011 – NOV 2013 : first green light for 1st pt to be included 2014

Vers la marchandisation des greffes de cellules et tissus ?

Les greffes de cellules, tissus et organes permettent de traiter des pathologies souffrant de déficiences organiques ou tissulaires ou, pour les greffes de moelle osseuse, d’exercer un effet immunothérapeutique vis-à-vis de formes graves de cancers. Les Américains E.D. Thomas et J. Murray, Prix Nobel de médecine en 1990 pour, respectivement, leurs travaux sur les greffes de cellules de moelle osseuse et de rein, ont, récemment disparu, la milionième greffe de moelle osseuse a été célébrée ; la planète compte plus d’un demi-million de personnes vivantes ayant reçu ce traitement. Les progrès de la biologie devraient promouvoir l’essor des greffes de cellules et tissus en médecine régénérative. Dans les pays industrialisés et émergents, ces traitements sont accessibles aux patients grâce à l’intervention d’équipes travaillant au sein d’établissements de santé ; en France, il s’agit d’hôpitaux, souvent publics, ou de l’Etablissement français du sang. A l’inverse des approches pharmacologiques classiques, pour lesquelles la production et la commercialisation de médicaments sont assurées par des établissements pharmaceutiques appartenant à des sociétés mondiales, les industriels sont moins présents dans ce secteur et fournissent des dispositifs médicaux et des réactifs utilisés aux étapes de prélèvement ou de conservation. Ces approches médicales sont rarement harmonisées et font intervenir des opérateurs de petite taille, organisés à une échelle locale ou régionale : banques de tissus ou unités de thérapie cellulaire, dont les activités sont autorisées, en France, par l’Agence nationale de sécurité des médicaments et des produits de santé (ANSM).

Le législateur d’organisation en 2007 en France, avec l’introduction en Europe, depuis 2007, de réglementations nouvelles définissant une nouvelle classe de produits thérapeutiques appelés « médicaux de thérapie innovante » (MTI) dans la législation française. Comme leur nom l’indique, ces nouveaux produits thérapeutiques ont le statut de médicaments, ce qui les distingue des préparations de thérapie cellulaire ou tissulaire de type biologique, distingue ici du patient ou d’un donneur et associé à une variabilité interindividuelle significative. Justifient le maintien d’une organisation distincte telle qu’elle est actuellement en place.

À court terme, alors que la démonstration du « succès » des PTIC est couramment attribuée à une variabilité interindividuelle significative, les banques de tissus et unités de thérapie cellulaire françaises sont aujourd’hui en situation de concurrence détestable pour arriver à un consensus sur les modalités de production de produits sanguins et de médicaments dérivés du sang.
MATHEC
Autoimmune Diseases and cell therapy platform

Clinical experts
Working in tandem
ABM EBMT standards
Daily care SSC HSCT
Academic studies
Industry trials

Teaching
Web site
ESH, St Louis
Paramedical

Research
ASTIS, NSIC
ASTILASTID
ASTIC
MSC

NTIC: common procedures, EBMT, Data base Med A med

Coordinating center
St Louis (UH 04)
+ St Antoine (EBMT)
+ 10 French JACIE accredited centres +
SFGM -TC+ ABM +