Transplantation in SSc

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Disclosures

• From A to Z (everybody) but
• Abbott
• Actelion
• Amgen
• Astra Zeneca
• BMS
• Celgene
• Genentech
• Medimmune
• Merck
• Pfizer
• Roche
• UCB
• United Therapeutics
Skin thickness over disease duration:
- Early vs advanced...
- **DIFFUSE SSc**
- **LIMITED SSc**

- Early (≤ 2 years):
  - Limited SSc: Lung, heart, GI, kidney
  - Diffuse SSc: Early stage

- Intermediate (2-5 years):
  - Limited SSc: Intermediate stage

- Late (≥ 10 years):
  - Limited SSc: Late stage
  - Diffuse SSc: Pulmonary hypert., malabsorption

*Medsger T & Steen V, Systemic Sclerosis, 1995, p 51, Williams & Wilkins*
Changes in causes of SSc-related deaths over time

Time period:
- 1972-1976
- 1977-1981
- 1982-1986
- 1987-1991
- 1992-1996
- 1997-2001

Frequency (%)
- Scleroderma renal crisis
- PAH
- Interstitial lung disease

courtesy of Virginia Steen.
Clinical management of SSc

- Subset & stage of disease
- Vascular remodelling
- Antifibrotic

- Screening for visceral complications (baseline & F/U)

- Renal insufficiency
- Myositis
- Digital ulceration
- Gastro-oesophageal reflux

- Midgut disease
- Interstitial lung disease
- PAH

• Autologous stem cell transplant
• Donor stem cell (allogeneic)
• Mesenchymal cells
• Organ transplant
What does a stem cell transplant do?

Abstract

PURPOSE OF REVIEW: Provides an update of hematopoietic stem cell transplantation for systemic sclerosis from phase I/II studies and prospective randomized phase III trials, and introduces the concept of mesenchymal stem cells as potential therapy for autoimmune disease.

RECENT FINDINGS: Around 170 transplanted systemic sclerosis patients are registered in Europe. Most received autologous, peripheral blood derived hematopoietic stem cell transplantation. Treatment-related mortality has fallen to 2.5% in the controlled trials compared with 12.5% in the first report in 2002. Over one-third of patients have experienced sustained remission. Two prospective randomized phase III studies are active: the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial in Europe and the Scleroderma Cyclophosphamide Or Transplant (SCOT) trial in the USA. Both have similar selection criteria, endpoint and control arms, but the SCOT trial uses radiation and less cyclophosphamide. So far, no unexpected toxicity has occurred. Reports produced in the past 12 months show reduction of skin collagen and reversal of microvascular remodelling, years after transplant. Bone marrow-derived mesenchymal stem cells from systemic sclerosis patients show in-vitro immunomodulatory properties equal to healthy controls.

SUMMARY: Hematopoietic stem cell transplantation is currently being tested in prospective randomized controlled trials and appears to 'reset' autoimmunity in systemic sclerosis. Mesenchymal stem cells may have an immunomodulatory role in autoimmune disease.
Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial.


Abstract

IMPORTANCE: High-dose immunosuppressive therapy and autologous hematopoietic stem cell transplantation (HSCT) have shown efficacy in systemic sclerosis in phase 1 and small phase 2 trials.

OBJECTIVE: To compare efficacy and safety of HSCT vs 12 successive monthly intravenous pulses of cyclophosphamide.

DESIGN, SETTING, AND PARTICIPANTS: The Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trial, a phase 3, multicenter, randomized (1:1), open-label, parallel-group, clinical trial conducted in 10 countries at 29 centers with access to a European Group for Blood and Marrow Transplantation-registered transplant facility. From March 2001 to October 2009, 156 patients with early diffuse cutaneous systemic sclerosis were recruited and followed up until October 31, 2013.

INTERVENTIONS: HSCT vs intravenous pulse cyclophosphamide.

MAIN OUTCOMES AND MEASURES: The primary end point was event-free survival, defined as time from randomization until the occurrence of death or persistent major organ failure.

RESULTS: A total of 156 patients were randomly assigned to receive HSCT (n = 79) or cyclophosphamide (n = 77). During a median follow-up of 5.8 years, 53 events occurred: 22 in the HSCT group (19 deaths and 3 irreversible organ failures) and 31 in the control group (23 deaths and 8 irreversible organ failures). During the first year, there were more events in the HSCT group (13 events [16.5%], including 8 treatment-related deaths) than in the control group (8 events [10.4%], with no treatment-related deaths). At 2 years, 14 events (17.7%) had occurred cumulatively in the HSCT group vs 14 events (18.2%) in the control group; at 4 years, 15 events (19%) had occurred cumulatively in the HSCT group vs 20 events (26%) in the control group. Time-varying hazard ratios (modeled with treatment × time interaction) for event-free survival were 0.35 (95% CI, 0.16-0.74) at 2 years and 0.34 (95% CI, 0.16-0.74) at 4 years.

CONCLUSIONS AND RELEVANCE: Among patients with early diffuse cutaneous systemic sclerosis, HSCT was associated with increased treatment-related mortality in the first year after treatment. However, HCST conferred a significant long-term event-free survival benefit.
Update on stem cell transplantation for systemic sclerosis: recent trial results.

Naraghi K¹, van Laar JM.

Abstract
Systemic sclerosis (SSc) is a heterogeneous condition characterized by the deposition of excess collagen in skin and internal organs due to vasculopathy, immune activation, low grade inflammation, and fibrosis. Progressive diffuse cutaneous SSc with organ involvement has a poor prognosis. The employment of autologous hematopoietic stem cell transplantation (HSCT) as a means to escalate immunosuppressive therapy has resulted in rapid and sustained improvement of skin thickening and functional ability, stabilization of major organ function with some improvement of vital capacity in pilot studies, registry analyses, and the phase II ASSIST trial. Results from the phase III ASTIS trial corroborate these findings and show long-term survival benefit of HSCT. The ASTIS and SCOT trials will determine whether the benefits of HSCT outweigh the risks of serious adverse events including treatment-related mortality of around 6-10% and potential long-term complications. Better patient selection and safer transplant regimens may improve the outcome of HSCT for SSc.
Stem cell therapies for systemic sclerosis.

Cipriani P¹, Ruscitti P, Giacomelli R.

Abstract

The presence of autoimmune diseases, including Systemic Sclerosis (SSc), suggest failure of the normal immune regulatory processes leading to activation and expansion of autoreactive effector immune cells. Recently, stem cell transplantation emerged as a novel rescue therapy for a variety of refractory autoimmune diseases. The therapeutic strategy involves the ablation of the aberrant self-reactive immune cells by chemotherapy and the regeneration of a new self-tolerant immune system formed by the transplanted stem cells. In the last few years, thousands of patients worldwide have received haematopoietic stem cell transplantation (HSCT), mostly autologous, as treatment for severe irreversible autoimmune diseases, with promising results. Here we review the results of published small series of SSc patients treated with allogeneic and autologous HSCT, as well as three randomized trials, exploring the safety and efficacy of autologous HSCT in SSc. Although the results are encouraging, nonetheless, the correct application of stem cell transplantation remains an area of active investigation. Results of larger randomized, double blind clinical trials, will certainly improve our knowledge of the appropriate clinical use of stem cell therapy in SSc patients.
**Abstract**

Systemic sclerosis is a rare disorder manifesting as skin and internal organ fibrosis, a diffuse vasculopathy, inflammation, and features of autoimmunity. Patients with diffuse cutaneous disease or internal organ involvement have a poor prognosis with high mortality. To date no therapy has been shown to reverse the natural course of the disease. Immune suppressive drugs are commonly utilized to treat patients, but randomized trials have generally failed to demonstrate any long-term benefit. In phase I/II trials, autologous hematopoietic stem cell transplantation (HSCT) has demonstrated impressive reversal of skin fibrosis, improved functionality and quality of life, and stabilization of internal organ function, but initial studies were complicated by significant treatment-related mortality. Treatment-related mortality was reduced by better pre-transplant evaluation to exclude patients with compromised cardiac function and by treating patients earlier in disease, allowing selected patients the option of autologous HSCT treatment. There are currently three ongoing randomized trials of autologous HSCT for systemic sclerosis: ASSIST (American Systemic Sclerosis Immune Suppression versus Transplant), SCOT (scleroderma cyclophosphamide versus Transplant), and ASTIS (Autologous Stem cell Transplantation International Scleroderma). The results from these trials should clarify the role of autologous HSCT in the currently limited therapeutic arsenal of severe systemic sclerosis.
Non autologous transplantation

Marked improvement of severe progressive systemic sclerosis after transplantation of mesenchymal stem cells from an allogeneic haploidentical-related donor mediated by ligation of CD137L.
Christopeit M, Schendel M, Föll J, Müller LP, Keysser G, Behre G.

Treatment of severe progressive systemic sclerosis with transplantation of mesenchymal stromal cells from allogeneic related donors: report of five cases.
Keyszer G, Christopeit M, Fick S, Schendel M, Taute BM, Behre G, Müller LP, Schmoll HJ.
Mesenchymal cells

Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells). This phenomenon has been documented in specific cells and tissues in living animals and their counterparts growing in tissue culture.
Mesenchymal stem cells in SSc are abnormal

Scleroderma Mesenchymal Stem Cells display a different phenotype from healthy controls; implications for regenerative medicine.


Abstract

INTRODUCTION: Vascular involvement is a key feature of Systemic sclerosis (SSc). Although the pericytes/endothelial cells (ECs) cross-talk regulates vessels formation, no evidences about the pericytes contribution to ineffective angiogenesis in SSc are available. Recent findings showed similarities between pericytes and Bone Marrow Mesenchymal Stem Cells (BM-MSCs). Due to difficulties in pericytes isolation, this work explores the possibility to use BM-MSCs as pericytes surrogate, clarifying their role in supporting neo-angiogenesis during SSc.

METHODS: To demonstrate their potential to normally differentiate into pericytes, both SSc and healthy controls (HC) BM-MSCs were treated with TGF-β and PDGF-BB. The expression of pericytes specific markers (α-SMA, NG2, RGS5 and desmin) was assessed by qPCR, western blot, and immunofluorescence; chemioinvasion and capillary morphogenesis were also performed. Cell-sorting of BM-MSCs co-cultured with HC-ECs was used to identify a possible change in contractile proteins genes expression.

RESULTS: We showed that BM-MSCs isolated from SSc patients displayed an up-regulation of α-SMA and SM22a genes and a reduced proliferative activity. Moreover during SSc, both TGF-β and PDGF-BB can specifically modulate BM-MSCs toward pericytes. TGF-β was found interfering with the PDGF-BB effects. Using BM-MSCs/MVECs co-culture system we observed that SSc BM-MSCs improve ECs tube formation in stressed condition, and BM-MSCs, sorted after co-culture, showed a reduced α-SMA and SM22a gene expression.

CONCLUSIONS: BM-MSCs from SSc patients behave as pericytes. They display a more mature and myofibroblast-like phenotype, probably related to microenvironmental cues operating during the disease. After their co-culture with HC-MVECs, SSc BM-MSCs underwent to a phenotypic modulation which re-programs these cells toward a pro-angiogenic behaviour.
Bone marrow-derived mesenchymal stem cells from early diffuse systemic sclerosis exhibit a paracrine machinery and stimulate angiogenesis in vitro.


Author information

Abstract

OBJECTIVE: To characterise bone marrow-derived mesenchymal stem cells (MSCs) from patients with systemic sclerosis (SSc) for the expression of factors implicated in MSC recruitment at sites of injury, angiogenesis and fibrosis. The study also analysed whether the production/release of bioactive mediators by MSCs were affected by stimulation with cytokines found upregulated in SSc serum and tissues, and whether MSCs could modulate dermal microvascular endothelial cell (MVEC) angiogenesis.

METHODS: MSCs obtained from five patients with early severe diffuse SSc (SSc-MSCs) and five healthy donors (H-MSCs) were stimulated with vascular endothelial growth factor (VEGF), transforming growth factor β (TGFβ) or stromal cell-derived factor-1 (SDF-1). Transcript and protein levels of SDF-1 and its receptor CXCR4, VEGF, TGFβ(1) and receptors TβRI and TβRII were evaluated by quantitative real-time PCR, western blotting and confocal microscopy. VEGF, SDF-1 and TGFβ(1) secretion in culture supernatant was measured by ELISA. MVEC capillary morphogenesis was performed on Matrigel with the addition of MSC-conditioned medium.

RESULTS: In SSc-MSCs the basal expression of proangiogenic SDF-1/CXCR4 and VEGF was significantly increased compared with H-MSCs. SSc-MSCs constitutively released higher levels of SDF-1 and VEGF. SDF-1/CXCR4 were upregulated after VEGF stimulation and CXCR4 redistributed from the cytoplasm to the cell surface. VEGF was increased by SDF-1 challenge. VEGF, TGFβ and SDF-1 stimulation upregulated TGFβ(1), TβRI and TβRII in SSc-MSCs. TβRII redistributed from the cytoplasm to focal adhesion contacts. SSc-MSC-conditioned medium showed a greater proangiogenic effect on MVECs than H-MSCs. Experiments with blocking antibodies showed that MSC-derived cytokines were responsible for this potent proangiogenic effect.

CONCLUSION: SSc-MSCs constitutively overexpress and release bioactive mediators/proangiogenic factors and potentiate dermal MVEC angiogenesis.
Impaired endothelium-mesenchymal stem cells cross-talk in systemic sclerosis: a link between vascular and fibrotic features.


Abstract

INTRODUCTION: To assess if an impaired cross-talk between endothelial cells (ECs) and perivascular/multipotent mesenchymal stem cells (MSCs) might induce a perturbation of vascular repair and leading to a phenotypic switch of MSC toward myofibroblast in Systemic Sclerosis (SSc).

METHODS: We investigated different angiogenic and profibrotic molecules in a tridimensional matrigel assay, performing co-cultures with endothelial cells (ECs) and bone marrow derived MSCs from patients and healthy controls (HC). After 48 hours of co-culture, cells were sorted and analyzed for mRNA and protein expression.

RESULTS: ECs-SSc showed a decreased tube formation ability which is not improved by co-cultures with different MSCs. After sorting, we showed: i. an increased production of vascular endothelial growth factor A (VEGF-A) in SSc-MSCs when co-cultured with SSc-ECs; ii. an increased level of transforming growth factor beta (TGF-β) and platelet growth factor BB (PDGF-BB) in SSc-ECs when co-cultured with both HC- and SSc-MSCs; iii. an increase of TGF-β, PDGF-R, alpha smooth muscle actin (α-SMA) and collagen 1 (Col1) in both HC- and SSc-MSCs when co-cultured with SSc-ECs.

CONCLUSION: We showed that during SSc, the ECs-MSCs crosstalk resulted in an altered expression of different molecules involved in the angiogenic processes, and mainly SSc-ECs seem to modulate the phenotypic switch of perivascular MSCs toward a myofibroblast population, thus supporting the fibrotic process.
Mesenchymal stem cells (MSCs) from scleroderma patients (SSc) preserve their immunomodulatory properties although senescent and normally induce T regulatory cells (Tregs) with a functional phenotype: implications for cellular-based therapy.


Mesenchymal stem cell (MSC) production of angiogenic molecules when co-cultured with epithelial cells (ECs) from

Figure 1: Tubular-like structure formation in Matrigel after 48 hrs. (A) Expression of endothelial cell markers CD31 (PECAM-1) and CD144 by flow cytometry analysis. The histograms in each graph show the increase in fluorescence for each endothelial cell marker.
Endothelial progenitor cell (or EPC) is a term that has been applied to multiple different cell types that play roles in the regeneration of the endothelial lining of blood vessels. Despite the history and controversy, the EPC in all its forms remains a promising target of regenerative medicine research.

Endothelial progenitor cell-based therapy for pulmonary arterial hypertension.

Yang JX¹, Pan YY, Zhao YY, Wang XX.

Author information

Abstract

A growing body of evidence in animal models and clinical studies supports the concept that endothelial progenitor cell (EPC)-mediated therapy ameliorates pulmonary arterial hypertension (PAH) and thus may represent a novel approach to treat it. Conversely, several experimental findings suggest that EPCs may be involved in PAH pathogenesis and disease progression. These discrepant results confuse the application of EPC transplantation as an effective treatment strategy for PAH. To improve the study of EPC transplantation in PAH therapy, it is high time that we resolve this dilemma. In this review, we examine the pathobiological changes of PAH, the characteristics of EPCs, and the underlying mechanisms of EPC effects on PAH.
Organ transplantation in SSc

- Kidney – scleroderma renal crisis
- Lung – interstitial lung disease
- Lungs – pulmonary arterial hypertension

Studies show that lung transplantation in SSc has increased mortality early on compared to age and sex matched controls with interstitial lung disease (IPF) and idiopathic pulmonary arterial hypertension (iPAH).

But

The 5 year survival is the same so patients with SSc should not be denied transplantation just because they have a systemic disease.
### Key Fibrotic Pathways Important to SSc

<table>
<thead>
<tr>
<th></th>
<th>Pathway/Signalling Pathway</th>
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<tbody>
<tr>
<td>1</td>
<td>TGF beta pathway (c-abl pathway, Smad signalling via TGF pathway, Non-canonical TGF pathways)</td>
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<tr>
<td>2</td>
<td>Epigenetics (miRNA 29)</td>
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<td>3</td>
<td>WNT</td>
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<td>4</td>
<td>B-cells</td>
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<td>5</td>
<td>CTGF</td>
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<td>6</td>
<td>TH-17 mediated repertoire</td>
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<td>7</td>
<td>Interferon</td>
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<td>8</td>
<td>PDGF</td>
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<td>9</td>
<td>IL13</td>
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<td>10</td>
<td>CCL-2 (MCP 1)</td>
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<td>11</td>
<td>ET-1</td>
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<td>12</td>
<td>Small molecules such as kinases</td>
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<td>13</td>
<td>Interleukin 4</td>
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<td>14</td>
<td>FRA-2</td>
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<td>15</td>
<td>PPAR gamma</td>
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<td>16</td>
<td>5-HT2b</td>
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<td>17</td>
<td>JAK2</td>
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<td>18</td>
<td>LPA</td>
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<td>19</td>
<td>IL-8</td>
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<td>20</td>
<td>HIF</td>
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<tr>
<td>21</td>
<td>LTB4</td>
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**What are the key fibrotic pathways important to SSc?**
What are the key fibrotic pathways important to SSc?
What are the key vascular pathways important to SSc?

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<tbody>
<tr>
<td>1</td>
<td>Endothelial dysfunction (from VEGF, von Willebrand, VCAM, ICAM)</td>
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<tr>
<td>2</td>
<td>Hypoxia-reperfusion injury (ROS)</td>
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<td>3</td>
<td>EC injury mediated by immune cells and granzyme</td>
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<tr>
<td>4</td>
<td>Activation of platelets and coagulation cascade (Tissue injury)</td>
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<tr>
<td>5</td>
<td>ET-1</td>
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<tr>
<td>6</td>
<td>Pericyte biology</td>
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<tr>
<td>7</td>
<td>PDGF</td>
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<td>8</td>
<td>Nitric Oxide</td>
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<td>9</td>
<td>Prostacyclin</td>
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<td>10</td>
<td>RENIN ANG II</td>
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<td>11</td>
<td>Serotonin</td>
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<td>12</td>
<td>LPA</td>
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<td>13</td>
<td>TGF beta (TGF/BMP)</td>
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<td>14</td>
<td>LTB4</td>
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<td>15</td>
<td>uPA</td>
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<td>16</td>
<td>Thrombin</td>
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What are the key vascular pathways important to SSc?
<table>
<thead>
<tr>
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<th>Key Immunological Pathways Important to SSc</th>
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<tbody>
<tr>
<td>1</td>
<td>CD4+ T lymphocytes activation with predominant Th2 polarization (Th2 cytokines, pro-fibrotic IL4, IL13, IL6 production)</td>
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<tr>
<td>2</td>
<td>B cell activation</td>
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<td>3</td>
<td>Autoantibody formation</td>
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<tr>
<td>4</td>
<td>CD8+ T-cell mediated (lung)(T-cells)</td>
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<tr>
<td>5</td>
<td>Interferon (interferon type 1, interferon gamma pathway)</td>
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<td>6</td>
<td>INNATE IMMUNITY</td>
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<tr>
<td>7</td>
<td>Macrophage function (Macrophage M2)</td>
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<tr>
<td>8</td>
<td>Fibrocyte/fibroblast--B cell interactions</td>
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<td>9</td>
<td>T regulatory cells</td>
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<td>10</td>
<td>IL 17</td>
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<td>11</td>
<td>Adipokines</td>
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<td>12</td>
<td>TGF Beta</td>
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<td>13</td>
<td>TLR</td>
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<td>14</td>
<td>Activated tissue monocyte</td>
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<td>15</td>
<td>MCP 1</td>
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<td>16</td>
<td>NF-kappaB</td>
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<td>17</td>
<td>MCP2</td>
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<td>18</td>
<td>CRP</td>
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What are the key immunological pathways important to SSc?
What are the key immunological pathways important to SSc?
What are the key immunological pathways important to SSc?
Which organ system(s) should be targeted to improve mortality in SSc?
Which organ system(s) should be targeted to improve quality of life of patients suffering from SSc?
**SKIN**
- MD skin activity
- MRSS
- Patient interference skin last month

**Pulmonary**
- FVC% predicted
- Breathing VAS (SHAQ)

**GI**
- GI VAS (SHAQ)
- Body Mass Index

**Cardiac**
- See next page
- DLCO% predicted
- HRCT score

**Biomarker**
- To be determined based on the trial
- BNP/NT-ProBNP

**Renal**
- Renal Crisis

**Raynaud’s**
- Raynaud’s VAS (SHAQ)

**HRQOL**
- Pain VAS
- Fatigue (instrument to be determined)

**Global Assessment**
- Pt GA
- MD GA

**CRISS**
Conclusions

• Stem cell transplantation is not for everyone with SSc
  • It has a high mortality esp in the first year
  • It is not covered by most health authorities
  • It is not a cure
  • Selection of patients is important

• But stem cell transplantation gives us hope that other more selective treatment can improve skin, organs, function, survival

• We have clues that resetting the immune system can rapidly improve skin involvement

• Other transplantation such as mesenchymal cells needs properly designed studies