EFFETTO IMMUNOREGOLATORIO delle
CELLULE STROMALI MESENCHELIMALI:

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PROPERTIES of MESENCHYMAL STROMAL CELLS (MSCs)

- **Multipotent cells**
  capable of differentiation into several mesenchymal lineages

- **Enhancement of hematopoietic stem cell engraftment**

- **Immunosuppressive properties**

- **Remarkable expansion after ex vivo culture**
Functional Properties of hMSCs for control of tissue homeostasis

- **Home** to sites of injury/inflammation
- **Modulate** immune response
- **Inhibit** inflammation (paracrine signaling)
- **Promote** tissue repair
TISSUE REPAIR and CONTROL of INFLAMMATION

Potential clinical use of MSCs in regenerative medicine and tissue engineering:

• repair of bone and cartilage degenerative disorders
• myocardial repair
• IBD (Crohn’s disease)
• Acute kidney/hepatic injury
• Wound healing (including radiation injury)
• CNS or spinal cord injury, MS, ALS
Immunological properties of MSCs *IN VITRO*

- MSCs inhibit T-lymphocyte proliferation induced by mitogens;
- High numbers of MSCs inhibit lymphocyte proliferation in MLC;
- The inhibitory effect is not HLA-restricted;
- MSCs suppress the generation of CTLs in a dose-dependent manner;
- MSCs do not influence CTLs if added directly into the cytotoxic assay;
- MSCs do not interfere with the lysis of K562 by NK cells;
- MSCs impair the generation of APC;
- MSCs favour the generation of CD4+/CD25+/FoxP3+ T regulatory cells;
- MSCs and Cs-A and exert a synergic suppressive effect on *in vitro* activation of alloantigen-specific CTLs;
- Third-party MSCs are able to suppress allo-specific IgG, IgA and IgM antibody production *in vitro.*

- *Di Nicola et al. Blood 2002*
- *Maccario et al. Haematologica 2005*
- *Nauta and Fibbe. Blood 2007*
- *Krampera et al. Blood 2003*
Addition of both MSCs-FCS and MSCs-PL were able to inhibit alloantigen-induced lymphocyte proliferation.
Addition of both MSCs-FCS and MSCs-PL favor the differentiation of CD4+CD25+FoxP3+ T regs
MSCs and the INNATE IMMUNE SYSTEM

MSCs SKEW MONOCYTES towards an ANTI-INFLAMMATORY IL-10 producing by PRODUCTION of IL-6
MSCs and NEUTROPHILs:
Activated MSCs induce RECRUITMENT of NEUTROPHILs by a pathway dependent on MIF and IL-8 and MODULATE NEUTROPHIL FUNCTIONs (apoptosis, chemokine secretion)

Brandau S et al. J Leukoc Biol 2010

MIGRATION ASSAY
Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages.

Choi H et al. Blood 2011
Mesenchymal stromal cell ‘LICENSING':
a multistep process.
The inhibitory activity is not constitutively expressed by MSCs, but depends on the activation of T cells and MSC themselves.

The 3 general functional properties:
- immune-inhibitory cells
- antiapoptotic and supporting properties toward different cells types (at resting condition)
- APCs and pro-inflammatory cells.

Krampera M. Leukemia 2011
MSCs: SENSORS and SWITCHERS of INFLAMMATION

Anti-Inflammatory

High levels IFN-γ/TNF-α

MSC

MSC 2

TRL3 ligation
dsRNA

high levels IDO/NO/PGE2

TGF-β

CD4+CD25+FoxP3+ T cell

Bernardo and Fibbe, Cell Stem Cell 2013

Pro-Inflammatory

Low levels IFN-γ/TNF-α

MSC

MSC 1

TRL4 ligation
LPS

Low levels IDO/NO/PGE2

CXCL9/CXCL10
MIP-1α/MIP-1β/Rantes

Activated T Cell

CCR5
CXCR3
MSCs: SENSORS and SWITCHERS of INFLAMMATION

**Anti- Inflammatory**
- MSC
  - +IL-6
  - IDO, PGE2
  - CD206, CD163
- M0
  - CCL-18
- M2
- T Cell
  - CD4+CD25+FoxP3+ T cell
  - TGF-β, PGE2, sHLA-G
- MSC
  - +IL-6

**Pro- Inflammatory**
- MSC
  - -IL-6
  - TSG6
- M0
  - CD40L/IFN-γ/IL-1
- M1
  - CD86
  - IFN-γ, TNF-α
- T Cell
  - Activated T Cell
  - High levels IFN-γ/TNF-α
- MSC
  - -IL-6

Bernardo and Fibbe, Cell Stem Cell 2013
SWITCHING MECHANISMS of MSCs into PRO-/ANTI-INFLAMMATORY CELLS RELY on PRODUCTION of SOLUBLE MEDIATORS

**HUMANs**

*Human MSC Suppression Correlates With Cytokine Induction of Indoleamine 2,3-Dioxygenase and Bystander M2 Macrophage Differentiation*

Inhibition of IDO activity of MSCs suppresses their immunesuppressive action on T cells

*Francois et al. Mol Ther 2012*

**MICE**

*Mesenchymal stem cells: a double-edged sword in regulating immune responses*

MSCs promote T-cell activation when NO is INSUFFICIENT

*Li et al. Cell Death and Differentiation 2012*
Clinical-Grade MSCsProduced Under Various GMP Processes Differ in Their Immunomodulatory Properties: Standardization of Immune Quality Controls

The systematic use of quantitative and reproducible validation techniques highlights differences in immunological properties of MSCs produced using various clinical-grade processes.

Menard C, …and Krampera M, Stem Cells Dev 2013
Suggestions for the assessment of regulatory properties of human MSCs: the ISCT proposal

1. A standard immune plasticity assay should be implemented with IFN-γ ± TNF-α used as model \textit{in vitro} priming agent.

2. Functional analysis of an expanded cell product may provide mechanistic insights on intra-study and inter-study variance in clinical response among patients.

3. The use of \textit{purified responders} would be widely practicable and should provide more generalizable guidance on relative functional potency of MSCs and as a companion to clinical trials.

4. Interrogating the \textit{IDO response} as part of an \textit{in vitro} licensing assay should be considered central.

5. Conclusions drawn on the basis of \textit{xenorecipient animal models} on how to conduct clinical trials should be drawn with caution.

6. The prospective hypothesis-driven analysis of lymphocyte populations in patient groups treated with MSC should be encouraged.

7. Clinical analysis should also include the monitoring of whether injected MSCs are the target of an immune response.

\textit{Krampera M, et al. Cytotherapy 2013}
Comparative Study of Immune Regulatory Properties of Stem Cells Derived from Different Tissues:

immunomodulation is not a peculiar feature of MSC-like cells, but actually a general property of SCs that may be induced or enhanced by inflammatory stimuli.

Human SC inhibitory effect on stimulated immune effector cell (IEC) proliferation: bone marrow-MSCs, olfactory ectomesenchymal SCs, leptomeningeal SCs, amniotic fluid SCs, cardiac SCs, and lung SCs

Di Trapani M, ...and Krampera M, Stem Cells Dev 2013
Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

*Le Blanc K et al. The Lancet 2004; 363: 1439-1441*
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 Bernardo, Mats Rembrger, Giorgio Dini, R Maarten Egeler, Andrea Bacigalupo, Willem Fibbe, 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 1.4×10^6 (min–max range 0.4–9×10^6) 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=69). 30 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.
# Patient characteristics

<table>
<thead>
<tr>
<th>Description</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>55 (100%)</td>
</tr>
<tr>
<td><strong>Gender: M / F</strong></td>
<td>34 / 21 (62% / 38%)</td>
</tr>
<tr>
<td><strong>Median age (years, and range)</strong></td>
<td>22 (0.5 – 64)</td>
</tr>
<tr>
<td><strong>Diagnosis:</strong></td>
<td></td>
</tr>
<tr>
<td>Haematological malignancies</td>
<td>43 (78%)</td>
</tr>
<tr>
<td>Non-malignant disorders</td>
<td>10 (18%)</td>
</tr>
<tr>
<td>Solid Tumors</td>
<td>2 (4%)</td>
</tr>
<tr>
<td><strong>Stem cell source:</strong></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>19 (35%)</td>
</tr>
<tr>
<td>PBSC</td>
<td>31 (56%)</td>
</tr>
<tr>
<td>CB</td>
<td>5 (9%)</td>
</tr>
<tr>
<td><strong>Type of donor:</strong></td>
<td></td>
</tr>
<tr>
<td>HLA-identical sibling</td>
<td>19 (35%)</td>
</tr>
<tr>
<td>HLA-identical UD</td>
<td>30 (55%)</td>
</tr>
<tr>
<td>HLA-partially matched donor</td>
<td>6 (10%)</td>
</tr>
</tbody>
</table>

*Le Blanc et al. Lancet 2008*
## Indications for MSC treatment

**Therapy-refractory grades II-IV acute GVHD**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>26</td>
</tr>
<tr>
<td>IV</td>
<td>24</td>
</tr>
</tbody>
</table>

**Previous failed therapy**

- First line: 55
- Second line: 33
- Third line: 14
- Fourth line: 4
- Fifth line: 2

**MSC treatment:** 92; **3rd party HLA-mismatched donors:** 69
Mesenchymal stem cell dose
Median 1.4 (range 0.4 – 9) x 10^6 MSC/kg

<table>
<thead>
<tr>
<th>Number of infusions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One infusion</td>
<td>27</td>
</tr>
<tr>
<td>Two doses</td>
<td>22</td>
</tr>
<tr>
<td>Three doses</td>
<td>4</td>
</tr>
<tr>
<td>Four doses</td>
<td>1</td>
</tr>
<tr>
<td>Five doses</td>
<td>1</td>
</tr>
</tbody>
</table>
Responses in patients with steroid resistant grade II-IV acute GVHD

- Complete response, disappearance of all symptoms: 30
- Improvement, partial response: 9
- Stable disease: 3
- Progressive disease: 13
- Overall response: 39/55 (71%)
Responses in pediatric patients (n=25)

- Complete response, disappearance of all symptoms 17
- Improvement, partial response 4
- Stable disease 2
- Progressive disease 2
- Overall response 21/25 (84%)
Cumulative incidence of TRM in patients who did or did not respond to MSC infusions

Figure 3: 1-year cumulative incidence of transplantation related mortality from time of infusion of mesenchymal stem cells. Transplantation related mortality was 37% (95% CI 19-55%) among the complete responders and 72% (55-89%) among the partial responders or non-responders.
1- and 2-year probability of OS in patients who did or did not respond to MSC infusions

Figure 2: Survival from time of haemopoietic-stem-cell transplantation in patients given mesenchymal stem cells. Survival at the end of follow-up was 52% (95% CI 34-70%) for the 30 complete responders and 16% (0-32%) for the 25 partial responders or non-responders.
Multiple infusions of MSCs induce sustained remission in CHILDREN with steroid-refractory, grade III-IV acute graft-versus-host disease.

- 37 children (age 3 months-17 years)
- Steroid-refractory aGvHD grade III-IV
  - 1 organ n=3
  - 2 organ n=11
  - 3 organ n=23
- >50% was histologically confirmed
- 2 centers common expansion protocol
  - Leiden Netherlands (n = 18)
  - Pavia Italy (n=19)
MSC characteristics

• 2 infusions (range 1-13) were administered

• 30 received more than a single dose
  – 12 /30 CR with single dose; 2nd MSC at time of initial steroid reduction

• median cell dose of 2x10^6/kg (range 0.9-3.0).

• 3rd party HLA-mismatched donors (n=31)

• Haploidentical relative (n=3)

• or both (n=3).

Ball, Bernardo et al. BJH 2013
PROBABILITY of OS for CHILDREN with CR after MSC TREATMENT is SIGNIFICANTLY SUPERIOR to that of CHILDREN with PR/NR

CR = 65% (38-82)

PR/NR = 0%

P = 0.00

Ball L.M. et al. Figure 1

Ball, Bernardo et al. BJH 2013
CUMULATIVE INCIDENCE of TRM for CHILDREN with CR after MSC TREATMENT is SIGNIFICANTLY INFERIOR to that of CHILDREN with PR/NR

Ball, Bernardo et al. BJH 2013
THERE IS a TREND for a LOWER TRM in CHILDREN with EARLY MSC TREATMENT as compared with CHILDREN with LATE MSC TREATMENT.
Conclusions from clinical trials in aGvHD

- Infusion of MSCs appeared to be safe and no major toxicities were observed.

- MSCs derived from BM may provide an effective therapy for patients with severe aGvHD who do not respond to treatment with corticosteroids.

- Treatment with MSCs resulted into a significant difference in survival and TRM between complete responders and partial and non-responding patients.

- Early treatment offers better results

RANDOMIZED TRIAL is ready for enrollment
Treatment of severe steroid-refractory acute GvHD with mesenchymal stromal cells. A phase III randomized double-blind multi-center HOVON study

Steroid-refractory Acute Graft-versus-Host Disease Grade II-IV (with gut and/or liver involvement)

Arm A

MMF + Placebo Day 1 and Day 8

Arm B

MMF + MSC 2x10^6/kg i.v. Day 1 and Day 8

Evaluation Day 29

Off protocol treatment
OSIRIS: company-driven randomized trial on the use of MSCs for patients with steroid-resistant aGvHD

- In a 2:1 randomization, 163 patients received multiple infusions of third-party MSCs and 81 were given placebo.

- Patients randomized to receive MSCs were given 8 infusions of the cells.

- Infusional toxicity, infection rates, and incidence of recurrent malignancy were similar in the two arms.

- No difference was observed in achieving the primary end point of a durable complete response for 28 days (35 vs. 30%), although there was a trend in favour of MSCs for patients with visceral involvement.

Martin P, et al. ASH Meeting 2009
OPEN BIOLOGICAL and CLINICAL QUESTIONS

✓ To what extent the described pathways are operational \textit{in vivo}? 
✓ There is a clear need to develop \textit{animal models} that address the interplay between MSCs and the host environment 
✓ Lack of \textit{biomarkers} predictive of response. 
✓ The beneficial effect of MSC infusion remains to be proved in \textit{randomized clinical trials} 
✓ How about \textit{dose and timing} of administration to obtain the optimal effect? Does this depend entirely on the local inflammatory conditions in the host? 
✓ How many MSC infusions should be administered? 
✓ Is there, if any, an ideal immune-suppressive agent to be used in combination with MSCs? 

MSCs warrant extensive further study
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