Bone marrow stroma: biology and therapeutic exploitation

Francesco Dazzi

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1. Bone marrow stroma and the physiology of the haemopoietic niche

2. The malignant niche: potentials for therapeutic intervention?

3. Mesenchymal stromal cells for HSCT complications
MSC induce the differentiation of GMP and CMP into macrophages

A

B

C

Sfp1 (PU.1)

Relative fold expression

0.0

0.5

1.0

1.5

2.0

2.5

CMP before

CMP after

GMP before

GMP after

egr1

Relative fold expression

0

20

40

50

60

CMP before

CMP after

GMP before

GMP after

mafb

Relative fold expression

0

200

400

600

800

CMP before

CMP after

GMP before

GMP after

cmaf

Relative fold expression

0

2000

4000

6000

8000

CMP before

CMP after

GMP before

GMP after
MSC-induced macrophages exhibit a ‘tissue healing’ profile

M1 markers

- **Cox2**: Relative fold expression
- **Ido**: Relative fold expression
- **Il12b**: Relative fold expression
- **Marco**: Relative fold expression
- **Nos2**: Relative fold expression

M2 markers

- **Arg-1**: Relative fold expression
- **Chia**: Relative fold expression
- **Ccl12**: Relative fold expression
- **Ir4**: Relative fold expression
- **Fizz1**: Relative fold expression

Legend:
- CD11b⁺ - BM
- CD11b⁺ - BM+MSC
- CD11b⁺ - BM+GM-CSF+IL-6
Imatinib produces complete remissions in most CML patients
MSC protect CML cells from imatinib via CXCL-12/CXCR4

Vianello et al. Haematologica 2010
AMD3100 sensitises APL to Ara-C.
Leukaemia boosts MSC induced myeloid generation

Macrophages (CD68+ CD14+ CD206+ CD163+ HLA-DR-)

MDSC (Lin− CD33+ CD11b+ HLA-DR−)
Conclusions

• Bone marrow stroma regulates self-renewal and differentiation of HSC
• Niches are different and consist of various components
• The bone marrow niches are hijacked by the leukaemia process
Mesenchymal stromal cells

Bone

Cartilage

Fat tissue
MSC

**Functions**

- Tissue support
  - 3D structure and mechano-transduction
- Tissue homeostasis
  - Modulation of inflammation
  - Regulation of stem cell renewal

**Therapeutic applications**

- Ex-vivo expansion of HSC
- Treatment of inflammatory conditions
- Improve tissue repair
- Manipulate the malignant niche
MSC can promote haemopoietic reconstitution

Jaganathan, Dazzi, Bonnet Leukemia 2010
The therapeutic effect is not associated with donor cell engraftment.
The MSC ‘facilitating effect’ does not seem to be related to haemopoietic engraftment
'MSC'

- Stemness
- Niche activity
- Immuno-modulation
Mesenchymal stromal cells exhibit immunosuppressive activities

Krampera et al, Blood 2003
<table>
<thead>
<tr>
<th>MSC have ‘anti-inflammatory’ activity</th>
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<tbody>
<tr>
<td>• Renal ischaemia</td>
</tr>
<tr>
<td>• Lung fibrosis</td>
</tr>
<tr>
<td>• Toxic liver injury</td>
</tr>
<tr>
<td>• Stroke</td>
</tr>
<tr>
<td>• Myocardial infarction</td>
</tr>
<tr>
<td>• Arthritis</td>
</tr>
<tr>
<td>• Multiple sclerosis</td>
</tr>
<tr>
<td>• IBD</td>
</tr>
<tr>
<td>• Sepsis</td>
</tr>
<tr>
<td>• Asthma</td>
</tr>
</tbody>
</table>
Aminoacid starvation is the main effector mechanism.

MSC

ARG NOS IDO HDC

Arginine Tryptophan Histidine

T cells (HSC?)

mTOR?

GCN2?

Cell cycle arrest

?
The inhibitory effect is not dependent on MHC class I expression.
MSC increase survival of aGVHD patients

Le Blanc et al, Lancet 2008
Incidence and severity of GVHD after prophylactic administration of MSC

<table>
<thead>
<tr>
<th></th>
<th>BM</th>
<th>PBSC</th>
<th>MSC + BM/ PBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGVHD &gt; II</td>
<td>25%</td>
<td>29%</td>
<td>28%</td>
</tr>
<tr>
<td>cGVHD lim</td>
<td>42%</td>
<td>22%</td>
<td>40%</td>
</tr>
<tr>
<td>cGVHD ext</td>
<td>21%</td>
<td>21%</td>
<td>19%</td>
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Lazarus et al, BBMT 2005
## Adults Data

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>21</td>
</tr>
<tr>
<td>Age (y) Median (Range)</td>
<td>49.2 (25-70)</td>
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<tr>
<td>Sex Male</td>
<td>17</td>
</tr>
<tr>
<td>Sex Female</td>
<td>4</td>
</tr>
<tr>
<td>Disease AML</td>
<td>6</td>
</tr>
<tr>
<td>Disease ALL</td>
<td>3</td>
</tr>
<tr>
<td>Disease MDS</td>
<td>2</td>
</tr>
<tr>
<td>Disease NHL/HL/MM</td>
<td>7</td>
</tr>
<tr>
<td>Disease Others</td>
<td>3</td>
</tr>
<tr>
<td>Number of Doses</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Dose (x10^6/Kg) Mean (Range)</td>
<td>3.6 (1.6-8.1)</td>
</tr>
</tbody>
</table>

## Children Data

<table>
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</thead>
<tbody>
<tr>
<td>Total (n)</td>
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<tr>
<td>Age (y) Median (Range)</td>
<td>7.3 (1-15)</td>
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<tr>
<td>Sex Male</td>
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<tr>
<td>Sex Female</td>
<td>9</td>
</tr>
<tr>
<td>Disease AML</td>
<td>7</td>
</tr>
<tr>
<td>Disease ALL</td>
<td>1</td>
</tr>
<tr>
<td>Disease MDS</td>
<td>1</td>
</tr>
<tr>
<td>Disease Aplastic Anemia/Other Anemias</td>
<td>3</td>
</tr>
<tr>
<td>Disease Others</td>
<td>4</td>
</tr>
<tr>
<td>Number of Doses</td>
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<tr>
<td>1</td>
<td>2</td>
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<td>2</td>
<td>3</td>
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<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Dose (x10^6/Kg) Mean (Range)</td>
<td>3.7 (1.7-7.4)</td>
</tr>
</tbody>
</table>

Dazzi and Apperley
Overall Outcome

Adults

Children
Adults

- No Response: 0%
- Response: 100%

Children

- No Response: 0%
- Response: 100%

Outcome after each infusion

- ≤2.5x10^6/Kg: 0%
  - n=7
- >2.5x10^6/Kg: 100%
  - n=34

- ≤2.5x10^6/Kg: 0%
  - n=10
- >2.5x10^6/Kg: 100%
  - n=33
MSC for GVHD: the need for ‘licensing’

Tissue affected by GVHD

DC

PC

T

MSC

IFN-γ

Chemokines

1

2

3

4

5

POSTCAPILLARY VENULE

Dazzi & Marelli-Berg, EJI 2008
The efficacy of MSC on GVHD depends on time of infusion

Polchert et al, EJL 2008
In vivo macrophage depletion impairs MSC therapeutic effect on GvHD
Clinical responses to MSC

- Acute inflammation
  - aMSC
  - M1

- Chronic inflammation
  - iMSC
  - M2

- Fibrosis
  - Persistent antigen stimulation
  - Th2
  - Th1

- Licensing
- Alternative licensing

Dazzi, Best Practice Haem 2011
Graft-versus-host disease

Acute GvHD

- Acute inflammation
- Alloimmunity
- Necrotic changes
- Confined to specific tissues
- Th1

Chronic GvHD

- Chronic inflammation
- Autoimmunity
- Fibrosis
- Multisystemic
- Th2
Pilot study

Total of 40 patients over 2 years

Grade II (visceral) GvHD

- 7 Clinical Centres (KCL-Marsden-ICL-Southampton-RFH-Bristol)
- KCL-Imperial for cell manufacturing
Primary aim of the study:

Identify a biomarker predictive of response to MSC

Responders  Non-Responders

Transcriptomic profile in peripheral blood monocytes
Conclusions

• MSC exhibit a potent anti-inflammatory effect

• Such an effect requires a conducive inflammatory environment

• Treatment of acute GvHD with MSC results into a significant clinical benefit

• A stratified approach is likely to produce better results