Mesenchymal stem cells therapy for type 1 diabetes

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Mesenchymal stem cells (MSC)

- MSC are multipotent stromal cells that can be isolated virtually from all tissues

- More than a century ago, the presence of progenitor cells in the bone marrow with the capability of differentiating to bone were identified

- By low-density culturing of bone marrow on plastic culture dishes, Friedenstein was able identify plastic-adherent colony-forming unit fibroblasts, which were later introduced largely by Owen as mesenchymal stromal cells
Characterization of MSC

**CFU-f and Morphology**
(Methylene blue staining, Electron Microscopy)

**Growth Curve**
(MTT staining)

**Differentiation Potential**
(Osteo-, Chondro-, Adipo-genesis)

**Cytokine profile**
(Real Time PCR, ELISA, ELISpot, Luminex)

**Surface Markers**
(FACS analysis, Immunohistochemistry)
MSC are characterized as fibroblast-like cells containing small amount of Golgi apparatus rough endoplasmic reticulum and mitochondria.
## FACS markers to characterize MSC

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Description</th>
<th>Expressed by</th>
<th>MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD105</td>
<td>Endoglin</td>
<td>Endothelial, BM cell subset, macrophages</td>
<td>+</td>
</tr>
<tr>
<td>CD29</td>
<td>β1 integrin</td>
<td>Leukocytes, fibroblasts, endothelial, epithelial</td>
<td>+</td>
</tr>
<tr>
<td>CD44</td>
<td>Cell adhesion molecule</td>
<td>Broad, memory T cells</td>
<td>+</td>
</tr>
<tr>
<td>CD34</td>
<td>Hematopoietic progenitor cell antigen</td>
<td>Hematopoietic precursors, capillary endoth, BM stroma, mast cells</td>
<td>-/+</td>
</tr>
<tr>
<td>CD45</td>
<td>Leukocyte common antigen</td>
<td>Leukocytes, immature erythrocytes</td>
<td>-</td>
</tr>
<tr>
<td>CD73</td>
<td>Ecto-5’-Nucleotidase</td>
<td>B/T cell subset, endothelial, epithelial, FDC, fibroblasts, neurons</td>
<td>+</td>
</tr>
<tr>
<td>CD106</td>
<td>Vascular cell adhesion molecule-1 (VCAM-1)</td>
<td>Endothelial, FDC, BM myeloid</td>
<td>+</td>
</tr>
<tr>
<td>CD166</td>
<td>Activated leukocyte cell adhesion molecule (ALCAM)</td>
<td>Neurons, T&lt;sub&gt;act&lt;/sub&gt;, monocytes, epithelial, fibroblasts</td>
<td>+</td>
</tr>
<tr>
<td>CD90.2</td>
<td>Thymocyte differentiation antigen-1b (Thy1.2)</td>
<td>Thymocytes, HSC, T cells</td>
<td>-/+</td>
</tr>
<tr>
<td>Sca-1</td>
<td>Stem cell antigen-1</td>
<td>Hematopoietic precursors</td>
<td>+/-</td>
</tr>
</tbody>
</table>
Proliferative capacity of MSC

Day 0

Day 3

Day 6

Day 9
MSC’s capacity to differentiate

<table>
<thead>
<tr>
<th>MSC</th>
<th>Chondrocytes</th>
<th>Adipocytes</th>
<th>Osteocytes</th>
</tr>
</thead>
</table>

C57BL/6 MSC
MSC clinical application

- Tissue engineering
- Transplantation
- GVHD
- Inhibition of tumor growth
- Tissue regeneration
- Autoimmune disorders
MSCs suppresses MLR response in a dose dependent manner
## MSC therapy in various disease models

<table>
<thead>
<tr>
<th>Model</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STZ diabetes</strong></td>
<td>Human-MSC grafted kidney and pancreas in STZ NOD.SCID mice ameliorating diabetes and kidney disease</td>
</tr>
<tr>
<td><strong>Heart transplantation</strong></td>
<td>Allogenic rat-MSC injected iv migrated to the heart during chronic rejection</td>
</tr>
<tr>
<td><strong>Heart transplantation</strong></td>
<td><em>Allogenic rat-MSC co-injected with CSA accelerate rejection</em></td>
</tr>
<tr>
<td><strong>Myocardial infarction</strong></td>
<td>Syngeneic rat-MSC showed an anti-inflammation role in ischemic heart disease.</td>
</tr>
<tr>
<td><strong>Acute lung injury</strong></td>
<td>Syngeneic intrapulmonary murine-MSC decreases the severity of endotoxin-induced acute lung injury and improves survival in mice</td>
</tr>
<tr>
<td><strong>Arthritis</strong></td>
<td>Allogenic murine-MSC reduce joint inflammation and increase Tregs generation</td>
</tr>
<tr>
<td><strong>Kidney Ischemia reperfusion injury</strong></td>
<td>Syngeneic murine-MSC are helpful in the restoration of tubular epithelial cells with an anti-inflammatory effect</td>
</tr>
<tr>
<td><strong>Multiple sclerosis model (EAE)</strong></td>
<td>Syngeneic murine-MSC home to inflamed lymphoid tissues reducing disease progression</td>
</tr>
<tr>
<td><strong>GHVD</strong></td>
<td>Allogenic rat-MSC prevent lethal GVHD</td>
</tr>
<tr>
<td><strong>BM transplantation</strong></td>
<td><em>Donor-MSC increase rejection of allogeneic donor BM cells</em></td>
</tr>
</tbody>
</table>
**MSC and clinical trials**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Condition/Therapy</th>
<th>Site/Sponsor</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mesenchymal Stem Cell Infusion as Prevention for Graft Rejection and GVHD</td>
<td>Hematological Malignancies, BM-derived donor MSC</td>
<td>University Hospital of Liege, Belgium</td>
</tr>
<tr>
<td>2.</td>
<td>Mesenchymal Stem Cells in Multiple Sclerosis (MSCIMS)</td>
<td>Multiple Sclerosis, BM-derived autologous MCS</td>
<td>University of Cambridge, UK</td>
</tr>
<tr>
<td>3.</td>
<td>Prochymal™ Adult Human Mesenchymal Stem Cells for Treatment of Moderate-to-Severe Crohn's Disease</td>
<td>Crohn's Disease, BM-derived allogeneic MSC (Prochymal™)</td>
<td>OsirisTherapeutics, USA</td>
</tr>
<tr>
<td>4.</td>
<td>Mesenchymal Stem Cell Infusion as Treatment for Steroid-Resistant Acute GVHD or Poor Graft Function</td>
<td>GVHD, BM-derived allogeneic MCS</td>
<td>University Hospital of Liege, Katholieke Universiteit Leuven, Belgium</td>
</tr>
<tr>
<td>5.</td>
<td>Evaluation of the Role of Mesenchymal Stem Cells in the Treatment of GVHD</td>
<td>GVHD, BM-derived donor MCS</td>
<td>Christian Medical College, India</td>
</tr>
<tr>
<td>6.</td>
<td>Treatment of Refractory GVHD by the Infusion of Expanded in-Vitro Allogeneic Mesenchymal Stem Cell</td>
<td>GVHD, BM-derived allogeneic MSC</td>
<td>University of Salamanca, Spain</td>
</tr>
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<td>7.</td>
<td>Extended Evaluation of PROCHYMAL™ Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease</td>
<td>Crohn's Disease, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
</tr>
<tr>
<td>8.</td>
<td>Efficacy and Safety of Adult Human Mesenchymal Stem Cells to Treat Patients Who Have Failed to Respond to Steroid Treatment for Acute GVHD</td>
<td>GVHD, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
</tr>
<tr>
<td>9.</td>
<td>Follow-up Study to Evaluate the Safety of Prochymal for the Treatment of GVHD Patients</td>
<td>GVHD, BM-derived allogeneic MSC</td>
<td>M.D. Anderson Cancer Center, TX, USA, Osiris Therapeutics</td>
</tr>
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<td>10.</td>
<td>Safety and Efficacy Study of Adult Human Mesenchymal Stem Cells to Treat Acute Gastrointestinal Graft Versus Host Disease</td>
<td>GVHD, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
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<td>11.</td>
<td>Donor Mesenchymal Stem Cell Infusion in Treating Patients With Acute or Chronic GVHD After Undergoing a Donor Stem Cell Transplant</td>
<td>GVHD, BM-derived donor MSC</td>
<td>Case Comprehensive Cancer Center, USA, National cancer Institute, (NCI)</td>
</tr>
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<td>12.</td>
<td>Evaluation of PROCHYMAL™ Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease</td>
<td>Crohn's Disease, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
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<td>13.</td>
<td>Prochymal Infusion for the Treatment of Steroid-Refractory Acute GVHD</td>
<td>GVHD, BM-derived allogeneic MSC (Prochymal™)</td>
<td>M.D. Anderson Cancer Center, USA, Osiris Therapeutics</td>
</tr>
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<td>14.</td>
<td>Safety and Efficacy of Prochymal for the Salvage of Treatment-Refractory Acute GVHD Patients</td>
<td>GVHD, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
</tr>
<tr>
<td>15.</td>
<td>OTI-010 for Graft-Versus-Host Disease Prophylaxis in Treating Patients Who Are Undergoing Donor Peripheral Stem Cell Transplantation for Hematological Malignancies</td>
<td>Hematological Malignancies, BM-derived autologous MSC</td>
<td>Johnson Comprehensive Cancer Center, USA, National Cancer Institute (NCI)</td>
</tr>
<tr>
<td>16.</td>
<td>Efficacy and Safety of Prochymal™ Infusion in Combination With Corticosteroids for the Treatment of Newly Diagnosed Acute GVHD</td>
<td>GVHD, BM-derived allogeneic MSC (Prochymal™)</td>
<td>Osiris Therapeutics, USA</td>
</tr>
</tbody>
</table>
Schematic representation of plausible mechanisms by which MSC regulate immune responses. MSC can increase the percentage of regulatory T cells through production of cytokines imparting regulation or promoting the generation of regulatory DC producing IL-10. In addition, MSC could suppress effector T cells through various growth factors, inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1), PG, or indolamine 2,3-dioxygenase (IDO). MSC may engage in cell-to-cell contact through a variety of receptors with T and endothelial cells. MSC might also reduce the generation and differentiation of DC. Up-regulation of MHC Class II on MSC could lead to down-regulation of NK cell cytotoxicity and proliferation. Finally, MSC may also act through down-regulation of immunoglobulin production by B cells.
MSC and Type 1 Diabetes
Type 1 diabetes

- The incidence of T1D has been steadily increasing.
- T1D has become one of the most challenging health problems of the 21st century worldwide.
- There is no cure for T1D
- MSC therapy has emerged as a promising treatment modality for diseases with immune etiology, particularly given the increasing appreciation for the morbidity associated with immunosuppression.
MSC trial in T1D

- Joint effort by JDRF and Osiris is underway assessing the therapeutic efficacy of MSC in T1D patients.
Non obese diabetic mice
Allogeneic MSC prevents autoimmune diabetes in NOD mice
Allogeneic MSC confers pathological protection
Allogeneic MSC reverses autoimmune diabetes in NOD mice

Insulin pellet

BALB/c MSC-treated (n=8)

Control (n=6)

Days after treatment

Glucoe levels (mg/dl)

% of normoglycemic

P=0.002

H&E

CD3

B220

Control d14

BALB/c-MSC d14

BALB/c-MSC d90
Characteristics of BALB/c and NOD MSC
NOD and BALB/c MSC characterization

<table>
<thead>
<tr>
<th>Protein</th>
<th>BALB/c MSC</th>
<th>NOD MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD29</td>
<td>93 1%</td>
<td>86 4%</td>
</tr>
<tr>
<td>CD44</td>
<td>53 9%</td>
<td>68 6%</td>
</tr>
<tr>
<td>CD45</td>
<td>1 1%</td>
<td>1 1%</td>
</tr>
<tr>
<td>CD73</td>
<td>26 4%</td>
<td>43 11%</td>
</tr>
<tr>
<td>CD90.2</td>
<td>6 3%</td>
<td>1 1%</td>
</tr>
<tr>
<td>CD105*</td>
<td>67 7% (p=0.001)</td>
<td>24 4%</td>
</tr>
<tr>
<td>CD166</td>
<td>12 5%</td>
<td>23 3%</td>
</tr>
<tr>
<td>Sca-1</td>
<td>37 7%</td>
<td>36 6%</td>
</tr>
</tbody>
</table>

Isotype

Ab

\(\text{Isotype} \ Ab\)
NOD and BALB/c MSC characterization

Costimulatory molecules

BALB/c

- CD40
  - 4 1%

- CD80
  - 6 3%

- CD86
  - 1 1%

- OX40
  - 5 1%

PD-1

- 2 1%

PD-L1*

- 16 3%

PD-L2

- 5 3%

p=0.01

NOD

- CD40
  - 2 1%

- CD80
  - 1 1%

- CD86
  - 3 2%

- OX40
  - 2 1%

- PD-1
  - 3 2%

- PD-L1*
  - 3 2%

- PD-L2
  - 4 1%

- PD-L2
  - 2 1%

BALB/c -MSC

- 21 4%*

NOD-MSC

- 5.4 1%

IFN-γ induction of PD-L1

Isotype

Ab

PD-L1

Ab
Monitoring the trafficking of BALB/c- and NOD-MSC in NOD mice
Why BALB/c MSC loses its effect? Due to recognition by host alloimmune system resulting in their rejection?
Superiority of NOR MSC in preventing T1D vs. allogeneic BALB/C MSC in NOD mice

% Normoglycemic

Weeks after treatment

H&E CD3 FoxP3 B220 Insulin Glucagon

BALB/c MSC-treated (n=35) Control (n=29) NOR MSC-treated (n=20)
Congenic NOR MSC therapy reverses hyperglycemia in NOD mice
Measuring the life span of allogeneic MSC using hGH
Recognition of Allogeneic MSC by Host

[Graph showing hGH concentration (pg/ml) over days post injection of MSC for Syngeneic and Allogeneic conditions.]

- Days Post Injection of MSC
  - 0, 5, 10, 15
  - hGH concentration (pg/ml)
    - Syngeneic: solid line
    - Allogeneic: dashed line

[Graph showing hGH Concentration (pg/ml) over days post injection for Humanized NOD-Scid Mice.]

- Days post injection
  - 0, 5, 10, 15
  - hGH Concentration (pg/ml)
    - Humanized NOD-Scid Mice: line with error bars
Characterization of NOR MSC

H&E

CD34

CD29

CD44

CD105

Control medium        Differentiation medium

Osteogenesis        Chondrogenesis

CD29               CD44                 CD45                CD73

CD106                Sca-1

CD90.2             CD105

AA CD45

CD73

CD90.2

CD105

CD166

Sca-1

% of Max

80.6  4.8%

54.2  5.8%

1.53  1.2%

23.5  2.4%

3.7   0.31%

76.1  5.5%

19.13  7.68%

29.3  3.0%
Immunoregulatory function of NOR MSC

CD3/CD28 stimulation assay

Control +NOR MSC

CD3/CD28 stimulation assay

Number of NOR MSC added

\[ \frac{3}{2}-\text{thy} \text{midine incorporation} \]

Control +NOR MSC

Proliferation Index

Frequency of cytokine-producing cells

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control</th>
<th>+2x10^4 NOR MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-(\gamma)</td>
<td>1500</td>
<td>2500</td>
</tr>
<tr>
<td>IL-6</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>
PD-L1 and immunoregulatory function of NOR MSC

- **PD-1**
  - Graph showing % positive cells vs. IFN-γ concentration (ng/ml)

- **PD-L1**
  - Graph showing % positive cells vs. IFN-γ concentration (ng/ml)

- **PD-L2**
  - Graph showing % positive cells vs. IFN-γ concentration (ng/ml)

- **Thymidine Incorporation**
  - Bar graph comparing Control, +NT siRNA-MSC, and +PD-L1 siRNA-MSC

- **Copies/copy**
  - Bar graph comparing Control and +PD-L1 siRNA-MSC
NOR MSC promotes formation of regulatory DC

**A**

-anti-IL-6  +anti-IL-6  -anti-IL-6  +anti-IL-6

-MSC  +MSC

**B**

-anti-IL-6  +anti-IL-6  -anti-IL-6  +anti-IL-6

-MSC  +MSC

**C**

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>Fgf3L</th>
<th>M-CSF</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**D**

-anti-IL-6  +anti-IL-6

-MSC  +MSC
Ex vivo assessment of immune regulation of NOR MSC in treated NOD mice

IL-6

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Treated NOD mice</td>
<td>Untreated</td>
<td>Treated NOD mice</td>
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</tbody>
</table>

IL-10

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
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<tr>
<td>Untreated</td>
<td>Treated NOD mice</td>
<td>Untreated</td>
<td>Treated NOD mice</td>
</tr>
</tbody>
</table>

**CD11c^low^CD11b^-CD45R/B220^+ Ly-6c^-**

**CD11c^high^CD11b^-CD45R/B220^-**
Human MSC and T1D-Preclinical studies
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSC</td>
<td>Chondrocytes</td>
<td>Osteocytes</td>
<td>Adipocytes</td>
</tr>
<tr>
<td>PB-MSC</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>BM-MSC</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>CB-MSC</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>
HUMAN BONE MARROW MSC
FACS markers

CD29 89%
CD 73 96%
CD90 98%
CD166 90%
CD105 80%
CD49e 93%
CD44 90%

Data from Najib El-Haddad, TRC, BWH
HUMAN CORD BLOOD MSC
SURFACE STAINING

CD29  98%
CD 73  98%
CD90  93%
CD166  %
CD105  18%
CD49e  73%
CD44  99%

MS IgG1 PE 71.4
CD29 APC 71.1

RAT IgG2B PE 70.8
CD44 PE 72.7

Ms IgG1 FITC 63.5
CD105 FITC 71.3

MS IgG1 PE 58.5
CD49c PE 58

Ms IgG1 FITC 63.5
CD105 FITC 71.3
<table>
<thead>
<tr>
<th></th>
<th>PB</th>
<th>BM</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD29</td>
<td>99</td>
<td>91.3</td>
<td>99.7</td>
</tr>
<tr>
<td>CD73</td>
<td>97</td>
<td>92.7</td>
<td>97.5</td>
</tr>
<tr>
<td>CD90</td>
<td>98</td>
<td>92</td>
<td>97.6</td>
</tr>
<tr>
<td>CD105</td>
<td>21</td>
<td>62.3</td>
<td>47.3</td>
</tr>
<tr>
<td>CD166</td>
<td>81</td>
<td>61.3</td>
<td>76.5</td>
</tr>
<tr>
<td>CD49e</td>
<td>95.8</td>
<td>89.3</td>
<td>96.1</td>
</tr>
</tbody>
</table>
hPB-MSC suppress CD4 T cell proliferation

NEG CTRL

POS CTRL

0.5 x 10^3 MSC

1.0 x 10^3 MSC

10 x 10^3 MSC

20 x 10^3 MSC

50 x 10^3 MSC

35 MSC
PB-MSC

IFN-γ Concentration (pg/ml)

TNF-α Concentration (pg/ml)

IL-1α Concentration (pg/ml)

NEG CTRL  POS CTRL  5x10²  10x10²  20x10³  35x10³  50x10³

BM-MSC

IFN-γ Concentration (pg/ml)

TNF-α Concentration (pg/ml)

IL-1α Concentration (pg/ml)

NEG CTRL  POS CTRL  5x10²  10x10³  20x10³  35x10³  50x10³

PROINFLAMMATORY CYTOKINES
hPB-MSC suppress autoreactive T cells
Challenges of Developing Cell-based Therapy with MSCs in Humans
Variation in MSC characteristics and function

The degree to which MSC will differentiate or function *in vitro* varies among individuals.

It is not clear whether this variation is due to a different amount of "true" progenitor cells in the culture or variable differentiation capacities of individuals' progenitors.

The importance of Flow cytometry based-methods to sort specific population of MSC (i.e. STRO-1) to obtain more homogenous population with higher rate of proliferation remains to be explored.
Variation in surface markers on MSC from different sources

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Variation in the proliferation of MSC from different strains

Andrea Augello, TRC, BWH
Hurdle: Adequate cell numbers and route of administration

• Inadequate cell number- Ex vivo expansion.
  A) Role for growth factors to enhance expansion?
  B) Role for human platelet lysates or $\beta 2\mu g$ to replace fetal bovine serum to eliminate risks of contaminations of prion, virus, or immunological reaction against xenogenic serum antigens

• Systemic (peripheral, portal, coronary vein) vs. direct injection to organ or under the skin.

• Higher rate of formation of unwanted tissue and tumor with local injection?

• Which type of immunosuppression will have synergistic effect with MSC?
Hurdle: Standardization and quality control studies of MSCs

• Comparing MSCs isolated from different passages and even different sources under identical ex-vivo conditions with respect to their morphology, expansion characteristics, immuno phenotype, and the success rate to isolate the cells.

• Developing the fastest and most efficient way to identify best quality controlled MSCs for disease of interest such as T1D?, Optimizing screening test to study and compare anti-diabetes effects of MSCs?.

• Shedding light on defining optimal cycle and culture conditions of the MSCs to be used for therapy.
Hurdle; Auto vs. Allogeneic MSC

- Use of "Off-the-shelf" Mesenchymal Stem Cells
- Could injected allo MSC cause systemic reaction?
- Would then allo MSC require more injection?
- There is a need for new concepts/tools to control MSC survival following injection?
Hurdle: Life span and trafficking of MSCs in the host in vivo

- Life span of MSC injected- influencing our protocols in terms of the length and cycles of administration but also on the issue of tumorigenecity.

- 2) Examining MSCs trafficking in the host: Migrate into sites of injury, i.e. to lymphoid tissues. Role for chemokine 1
## Potential risks using MSC.

<table>
<thead>
<tr>
<th>Safety Profiling:</th>
<th>Consideration:</th>
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</thead>
<tbody>
<tr>
<td>Cytogenetic instability of MSC per se and support of tumor growth</td>
<td>Does auto-MSC pose equal risk as allo-MSC?</td>
</tr>
<tr>
<td>Ectopic MSC differentiation</td>
<td>Local vs. systemic administration?</td>
</tr>
<tr>
<td>Fetal calf serum response</td>
<td>Use of cocktails of growth factors or platelet lysate?</td>
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<tr>
<td>Effect of inflammatory molecules released from MSC</td>
<td>Could rejected allogeneic MSC cause systemic reactions?</td>
</tr>
<tr>
<td>Cell product purification</td>
<td>Highly pure vs. mixed population?</td>
</tr>
</tbody>
</table>
Summary remarks

- MSC based therapy holds much promise for the treatment of debilitating diseases.
- MSC have already been used in human to treat various diseases, however, there is a need for following studies for the disease of interest
  - Standardization and quality control
  - Characterization of MSC and their immunomodulatory function
  - Animal studies to better understand their trafficking, survival, and studies to optimize MSC therapy
  - Animal studies to elucidate their tumorogenicity if any?
  - Elucidating the differential immunomodulatory properties of human bone marrow, blood, and cord blood MSC
  - Developing biomarkers to monitor MSC immunosuppressive effect post injection
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