MSC

- Identified by Fridenstein in early 70’s
  - Adherent fibroblastic cells able to form colonies (CFU-F) and to differentiate into bone

- MSC
  - Rare cells expressing no specific marker
  - Found in the bone marrow, adipose tissue, and Wharton’s jelly (umbilical cord) + many tissues
  - Strong in vitro expansion (self-renewal)
  - Multipotency
  - Very few data on native MSC
Mesenchymal stem cells

Frenette Ann Rev Immunol 2013; 31:285

Ectopic organized hematopoietic bone
Native mesenchymal stem cells

CD146<sup>pos</sup>

CD45<sup>-</sup>-CD146<sup>+</sup>

CD45<sup>-</sup>-CD146<sup>-</sup>

MSCs

CD146<sup>+</sup> cells

CD146<sup>-</sup> cells

CD34

CD146

CD34

A

V

C

BM engraftment

Crisan *Cell Stem Cell* 2008, 3:301
Sacchetti *Cell* 2007, 131:324
Corselli *Blood* 2013, 121:2891
Native mesenchymal stem cells

Qian JBC 2012, 287:25795
Cultured MSC

- Self-renewal?
- Heterogeneity

Mesenchymal stromal cells

- Capacity of differentiation?
- Paracrine activity, immunosuppression/anti-inflammatory effect
Production of MSC

Numerous enrichment approaches (Stro-1, CD73, CD49a…) without current clinical application
Qualification of initial product

% $\text{CD}14^{\text{neg}} \cap \text{CD}45^{\text{neg}} \cap \text{CD}34^{\text{neg}} \cap \text{CD}235a^{\text{neg}} \cap \text{CD}73^{\text{pos}} \cap \text{CD}271^{\text{pos}}$ within viable cells

Nb of CFU/ml in the BM

CD73

CD45/CD235a/CD14

CD271

Collagenase

DNAse

CFU-F
Qualification of initial product

Correlation between CFU-F number and % of MSC

Correlation quantification par CMF ou par comptage des CFUF
Numerous clinical applications

- Immune-mediated diseases
  - Autoimmune diseases
  - aGVHD, organ transplant
  - Sepsis (mouse models)

- Regenerative medicine
  - Cardiovascular disease (cardiac muscle, vessels)
  - Epithelium (skin, cornea)
  - Skeletal tissue (long bone, cartilage, mandibula)
MSC for autoimmune disorders

Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study

Peter Connick,* Madhan Kolappan, * Charles Crawley, Daniel J Webber, Rickie Petani, Andrew W Michell, Bing Qing Du, Shi-Lu Luan, Daniel R Altmann, Alan J Thompson, Alastair Compston, Michael A Scott, David H Miller, Siddharthan Chandran

Lancet Neurol 2012; 11: 150-56

Findings We isolated, expanded, characterised, and administered mesenchymal stem cells in ten patients. The mean dose was 1.6 × 10⁶ cells per kg bodyweight (range 1.1–2.0). One patient developed a transient rash shortly after treatment; two patients had self-limiting bacterial infections 3–4 weeks after treatment. We did not identify any serious adverse events. We noted improvement after treatment in visual acuity (difference in monthly rates of change −0.02 logMAR units, 95% CI −0.03 to −0.01; p=0.003) and visual evoked response latency (−1.33 ms, −2.44 to −0.21; p=0.020), with an increase in optic nerve area (difference in monthly rates of change 0.13 mm², 0.04 to 0.22; p=0.006). We did not identify any significant effects on colour vision, visual fields, macular volume, retinal nerve fibre layer thickness, or optic nerve magnetisation transfer ratio.

Interpretation Autologous mesenchymal stem cells were safely given to patients with secondary progressive multiple sclerosis in our study. The evidence of structural, functional, and physiological improvement after treatment in some visual endpoints is suggestive of neuroprotection.
MSC for autoimmune disorders

Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn’s disease

Rachele Ciccoio,1 Maria Ester Bernardo,2,3 Adele Sgarella,4 Rita Maccario,2,5 Maria Antonietta Avanzini,2 Cristina Ubezio,1 Antonella Minelli,6 Costanza Alvisi,7 Alessandro Vanoli,8 Fabrizio Calliada,9 Paolo Dionigi,4 Cesare Perotti,10 Franco Locatelli,3 Gino Roberto Corazza1

**Results** MSC expansion was successful in all cases; sustained complete closure (seven cases) or incomplete closure (three cases) of fistula tracks with a parallel reduction of Crohn’s disease and perianal disease activity indexes (p<0.01 for both), and rectal mucosal healing were induced by treatment without any adverse effects. The percentage of mucosal and circulating regulatory T cells significantly increased during the treatment and remained stable until the end of follow up (p<0.0001 and p<0.01, respectively). Furthermore, MSCs have been proven to affect mucosal T cell apoptotic rate.

BM-MSC PL
2-5 intrafistular injections
10^6 MSC/cm^2
10 patients
MSC for alloimmune disorders

Induction Therapy With Autologous Mesenchymal Stem Cells in Living-Related Kidney Transplants
A Randomized Controlled Trial

Tan et al. JAMA 2012;307:1169

Results Patient and graft survival at 13 to 30 months was similar in all groups. After 6 months, 4 of 53 patients (7.5%) in the autologous MSC plus standard-dose CNI group (95% CI, 0.4%-14.7%; P = .04) and 4 of 52 patients (7.7%) in the low-dose group (95% CI, 0.5%-14.9%; P = .046) compared with 11 of 51 controls (21.6%; 95% CI, 10.5%-32.6%) had biopsy-confirmed acute rejection. None of the patients in either autologous MSC group had glucocorticoid-resistant rejection, whereas 4 patients (7.8%) in the control group did (95% CI, 0.6%-15.1%; overall P = .02). Renal function recovered faster among both MSC groups showing increased eGFR levels during the first month after surgery than the control group. Patients receiving standard-dose CNI had a mean difference of 6.2 mL/min per 1.73 m² (95% CI, 0.4-11.9; P = .04) and those in the low-dose CNI of 10.0 mL/min per 1.73 m² (95% CI, 3.8-16.2; P = .002). Also, during the 1-year follow-up, combined analysis of MSC-treated groups revealed significantly decreased risk of opportunistic infections than the control group (hazard ratio, 0.42; 95% CI, 0.20-0.85, P = .02)
MSC for alloimmune disorders

Ciclosporin
Prednisolone
Methylprednisolone
PUVA
Infliximab and decitabine

Allogeneic BM-MSC FCS
0.4-9 x 10^6/kg IV
55 patients

Table 4: GVHD response and outcome

<table>
<thead>
<tr>
<th></th>
<th>Children (n=25)</th>
<th>Adults (n=30)</th>
<th>All patients (n=55)</th>
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</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>17</td>
<td>13</td>
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</tr>
<tr>
<td>Partial response</td>
<td>4</td>
<td>5</td>
<td>9</td>
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<tr>
<td>Stable disease</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>2</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Overall response</td>
<td>21</td>
<td>18</td>
<td>39</td>
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<tr>
<td>Survival*</td>
<td>13</td>
<td>8</td>
<td>21</td>
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<tr>
<td>Limited chronic GVHD</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Extensive chronic GVHD</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

*At last data collection, March, 2007.

Figure 1: Clinical course and Immunosuppression of the patient
↓=mesenchymal stem-cell transplantation. ASCT=allogeneic stem-cell transplantation.
MSC=mesenchymal stem cells.

MSC for critical limb ischemia

Randomized clinical trial
Ixmyelocel-T (BM-derived MSC + macro, 12-d culture)
IM inj (20 points)

Figure 4 Time to first occurrence of treatment failure for all patients who had baseline wounds. Kaplan-Meier survival plot of time to treatment failure (major amputation of injected leg, all-cause mortality, doubling of total wound surface area from baseline, de novo gangrene) for post hoc analysis of patients who had baseline wounds. Censored observations.

Powell et al. Mol Ther 2012;6:1280
Common mechanisms of action

- Few evidence for cell replacement
  - Poor long-term persistence
  - Poor transdifferentiation capacities

- Touch-and-go paracrine effects
  - Trophic factors
  - Cell recruitment
  - Immunomodulatory & antiinflammatory
  - Vasculature improvement
In-vitro expanded MSC and immunology

- Survival
- Proliferation
- Differenciation

- TGFβ, sHLA-G
- IFN-γ
- TNF-α
- Tαβ

- IL-6, IL-10
- CCL2

- PGE2
- IL-6, IL-10

- IDO (iNOS, HO-1)
- sHLA-G
- PGE2
- IL-10
- TGFβ
- Galectins
- Jagged-1

- Survival
- Proliferation
- IFN-γ

- Adaptive Immunity
  - Monocyte
    - iDC
      - mDC
        - IL-12
          - T-cell activation

- Differenciation

- Maturation
General mechanisms of immunoregulation

- **Cytokines**: IL-10, TGFβ, HGF, VEGF...
- **Membrane molecules**: PD1/PD-L1, PD-L2, CD200R/CD200...
- **Enzymes**
  - IDO-1, IDO-2 (Trp)
  - iNOS & ARG-1 (Arg)
  - HO (heme)
- **PGE2**
- **HLA-G** (soluble and membrane forms)
**T-cell proliferation**

- Activated T cells
- Activated T cells + CSM
- Activated T cells + CSM + L-1MT
- Activated T cells + CSM + D-1MT

**Index de prolif**

<table>
<thead>
<tr>
<th>Condition</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
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<tbody>
<tr>
<td>T + antiCD3/28</td>
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</tr>
<tr>
<td>CSM + L-1MT</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CSM + D-1MT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
**MSC and B cells**

Support resting and activated B-cell survival
Maintain the proliferative potential of resting B cells
Support activated B-cell survival & proliferation

---

**ACTIVATION STEP**

**DIFFERENTIATION STEP**

B cells vs B + MSC

**Absolute number of viable B cells ($\times 10^5$)**

<table>
<thead>
<tr>
<th></th>
<th>B cells</th>
<th>B + MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28%</td>
<td>19%</td>
</tr>
</tbody>
</table>

***
MSC and B cells

Alter normal B-cell differentiation

Naive B cells

ACTIVATION

DIFFERENTIATION

CD38

CD20

% of CD138+ cells

MSC

n.s.

IgA

IgG

IgM

ASC per 10^6 B cells

BCL-6

PRDM1

IRF4

XBP1

MSC

B cells
MSC and B cells

Functional heterogeneity of MSC depending on the cytokine context
In-vitro expanded MSC and immunology

Macrophage

- IL-12
- TNF-α
- IL-10

Neutrophil

- IL-6
- TSG-6
- IDO
- PGE2
- sHLA-G

NK cells

- IFN-γ
- TNF-α

- Proliferation
- Cytotoxicity
- IFN-γ

- NK activated

KILLING

Survival

Migration

Burst oxidative

Survival

Proliferation

Cytotoxicity

IFN-γ
T-cell proliferation

Activated T cells

Activated T cells + CSM

Activated T cells + CSM + L-1MT

Activated T cells + CSM + D-1MT

NK-cell proliferation

T + antiCD3/28

CSM

CSM + L-1MT

CSM + D-1MT

NK + IL-2

CSM

CSM + L-1MT

CSM + D-1MT
MSC and immunoregulation

- Critical parameters
  - Species (mouse *versus* human)
  - Tissue origin
  - Culture conditions (PL *versus* FCS, FGF-2, whole BM *versus* BMMC...)
  - **Number of PD**
  - Validation of immunological assays

- STANDARDIZATION
MSC-related parameters

- Tissue origin
- Culture conditions
- Priming
- Culture expansion (PD, senescence)
- MSC to immune cell ratios

Donor-related variability
- Species

Read-out
- Viability
- Proliferative potential
- Stimulation cocktail
- Specific inhibitors

Cell purification

MSC to immune cell ratios

Immune cell-related parameters

MSC

PMN

MO

DC

NK

Th

CTL

B

Stim.

Sensebé Hum Gen Ther 2011
Ménard Stem Cell Dev 2013
Ménard Stem Cell Res Ther 2013
Krampera Cytotherapy 2013
Clinical-grade MSC & Immunoregulation

What is the best clinical-grade MSC?

Various processes

Various sources

- IDO
- NO
- HO
- PGE2
- TSG-6

Phenotype

PMN

TNF/IFN-γ

Tαβ

NK

B

Mo
MSC heterogeneity: production process

Clinical-grade MSC display various immune properties depending on the production process.
# MSC heterogeneity: production process

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>HLA DR</th>
<th>CD200</th>
<th>CD54</th>
<th>CD106</th>
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<td>1.4</td>
<td>8.4</td>
<td>1.4</td>
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<tr>
<td>PADSC-PL</td>
<td>1</td>
<td>1</td>
<td>8.6</td>
<td>5.5</td>
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<tr>
<td>BMMSC-PL</td>
<td>4.3</td>
<td>10.6</td>
<td>2.7</td>
<td>5.3</td>
</tr>
<tr>
<td>PBMMSC-PL</td>
<td>1</td>
<td>1</td>
<td>8.6</td>
<td>5.5</td>
</tr>
<tr>
<td>BMMSC-FCS</td>
<td>4.3</td>
<td>10.6</td>
<td>2.7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

**Heterogeneity and Production Process**

- **TNFAIP6**, a key marker for MSC heterogeneity, is significantly expressed in different cell types.
- The production process involves analyzing cell surface markers like HLA DR, CD200, CD54, and CD106.

The scatter plot and histogram data reflect the variability in expression levels across different cell types, highlighting the importance of standardized production processes for consistent outcomes in MSC therapies.
MSC heterogeneity: tissue of origin

Role of tissue origin?

Affymetrix microarrays

474 differentially expressed genes
276 upregulated in ADSC
198 upregulated in BM-MSC

TNF/IFN-γ

- IDO
- PGE2
- TSG-6
- HO
- NO

PMN

Mo

NK

B

Tαβ

Tαβ

Tαβ

B
MSC heterogeneity: tissue origin

**VCAM-1**

Fold change
ADSC/BM-MSC

0.076

**ICAM-1**

2.91

**MHC class II**

0.303

**HLA-DR**

**IDO**

**Fold change**
ADSC/BM-MSC

**MC heterogeneity: tissue origin**

**VCAM-1**

Fold change
ADSC/BM-MSC

0.076

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ADSC/BM-MSC

0.076

**ICAM-1**

2.91

**MHC class II**

0.303

**HLA-DR**

**IDO**

**Fold change**
ADSC/BM-MSC
MSC plasticity: Licensing by inflammatory context

Prevention of aGVHD in mice

- Untreated MSC
- MSC treated with 5U IFN-γ
- MSC treated with 50U IFN-γ
- MSC treated with 500U IFN-γ

MSC plasticity: Licensing by inflammatory context

- Influence of TLR ligands
  - Effect on MSC-mediated reprogramming of macrophages in sepsis

Safety of MSC uses

- **Short Term Safety**
  - donor / starting material
  - Processes
  - controls for release

- **Long term Safety**
  - transformation ?
  - Senescence ?
  - Favoring tumor growth ?
  - Unwanted homing & differentiation ?
Transformation of ADSC?

<table>
<thead>
<tr>
<th>Sample</th>
<th>MSC-TMC transition</th>
<th>Post-senescence MSC</th>
<th>TMC karyotype</th>
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</thead>
<tbody>
<tr>
<td>H</td>
<td>YES</td>
<td>ND</td>
<td>46,XY, der[5]5[p15q2], 81,XXYY, der[5]5[p15q2], der[1][3][1][q25]</td>
</tr>
<tr>
<td>14 EGFP</td>
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<td>45,XO, der[5]5[p15q2], der[1][3][1][q25]</td>
</tr>
<tr>
<td>14 EGFP2</td>
<td>YES</td>
<td>ND</td>
<td>45,XY (8)</td>
</tr>
<tr>
<td>16</td>
<td>NO</td>
<td>47,XY (8)</td>
<td>45,XY (8)</td>
</tr>
<tr>
<td>16 EGFP</td>
<td>YES</td>
<td>ND</td>
<td>45,XY (8)</td>
</tr>
<tr>
<td>16 EGFP2</td>
<td>YES</td>
<td>ND</td>
<td>46,XY</td>
</tr>
<tr>
<td>21</td>
<td>NO</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>NO</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>31</td>
<td>NO</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Final retraction: Cancer Res 2010;70 (16):6682

Contamination

Research Article

Human mesenchymal stem cell transformation is associated with a mesenchymal–epithelial transition

Daniel Rubio a, Silvia Garcia b, Teresa De la Cueva a, M a F. Paz b, Alison C. Lloyd d, Antonio Bernad b,e, Javier Garcia-Castro c
Transformation of BM MSC?

In this context, we reported, in a recent study, spontaneous transformation of hMSC observed in two independent laboratories (1).

The Issue of Cell Line Misidentification

Rosland GV et al Cancer Res 2009
MSC production & control: French experience

- Prevention of aGVHD post allo-HSCT using MSC (double blinded versus placebo)
- 74 patients, 22 centers
- Strong efforts in:
  - Standardization of MSC production and QC
  - Centralization of specialized MSC qualification
    - Immunogenicity, Immunosuppression
  - Centralization of monitoring
    - MSC engraftment (CFU-F chimerism by Q-PCR)
    - Immunological properties of MSC (in vitro, in vivo)
    - Immune reconstitution (Phenotype, PBMC proliferation)
<table>
<thead>
<tr>
<th>Culture protocol</th>
<th>Donor number (Age)</th>
<th>PD (Proliferation rate)</th>
<th>Karyotype P1</th>
<th>Karyotype P2</th>
<th>hTERT P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCS/FGF-2</td>
<td>1A (55 y)</td>
<td>23 (29.5)</td>
<td>46, XX [15]</td>
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<td>2A (38 y)</td>
<td>23 (209.2)</td>
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<td>46,XY [21]</td>
<td>Neg</td>
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<td>4A (29 y)</td>
<td>20 (145.8)</td>
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<td>5A (41 y)</td>
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<td>46, XY [16]</td>
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<td>21 (78.2)</td>
<td>49, XY, +5, +8, +20 [3]/46, XY [19]</td>
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<tr>
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<td>10A (28 y)</td>
<td>19 (39.8)</td>
<td>46, XY [27]</td>
<td>ND</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>12A (47 y)</td>
<td>22 (44.6)</td>
<td>47, XX, +5 [15]/46, XX [5]</td>
<td>ND</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>13A (56 y)</td>
<td>20 (54.2)</td>
<td>46, XY [20]</td>
<td>ND</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>12A2 (47 y)</td>
<td>20 (57.5)</td>
<td>47, XX, +5 [3]/46, XX [17]</td>
<td>47,XX,+5[2]/46,XX[28]/</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>13A2 (56 y)</td>
<td>17 (9.44)</td>
<td>46, XY [30]</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>16B* (23 y)</td>
<td>19 (16.5)</td>
<td>46,XY [30]</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

- Recurrent aneuploidy, not dependent on the culture process, donor effect?, growth disadvantage
MSC production & control: French experience

- **In vitro senescence**
  - Growth arrest at PD 35-52
  - No hTERT induction, No c-myc modulation
  - Induction of p16 between P4 et P7
  - β-Gal Staining

- No anchorage independent growth, no tumor in SCID mice

Karyotype is not relevant
Further study on MSC stability
Definition of guidelines at French and European levels
Impact of senescence

BM MSC = ADSC

Senescence affects immunoregulatory properties of MSC
Impact of senescence

Senescence affects immunoregulatory properties of MSC
Stromal cells and cancer

Direct & Indirect roles of CAF
- Cell growth
- Metastasis
- Angiogenesis
- Drug resistance
- Immune escape

MSC and FL

1. Stromal cells recruit and support directly malignant B cells
2. Malignant B cells confer a supportive phenotype to stromal cells
3. Stromal cells organize malignant B-cell niche (interactions with TAM, T cells...)

CXCL12
BAFF
Hh
TNF/LT
CCL2
IL-15/IL-15Rα
IL-15Rβ/γc
CD40
CD40L
TAM
TNF, LT
IFNγ
PD-1
TNF, LT
IFNγ

Ame-Thomas Blood 2007; 109:693
Guilloton Blood 2012; 119:2556
MSC and multiple myeloma

- Specific GEP (GDF-15, IL-6...)
- Increased expression of XBP1s
- Increased capacity to sustain MM cell growth
- Increased capacity to induce osteoclast formation
MSC and AML transformation

Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia

Marc H. G. P. Raaijmakers1,6,7*, Siddhartha Mukherjee1,2,6,7†, Shangqin Guo1,6,7, Siyi Zhang1,6,7, Tatsuya Kobayashi3, Jesse A. Schoonmaker1,6,7, Benjamin L. Ebert8,9, Fatima Al-Shahrour8,9, Robert P. Hasserjian4, Edward O. Scadden1,6,7, Zinmar Aung1,6,7, Marc Matza1,6,7, Matthias Merkenschlager10, Charles Lin5, Johanna M. Rommens11 & David. T. Scadden1,2,6,7

“Niche-induced oncogenesis”
Origin
Mesoderm vs neuroepithelium

Stem Cells
Self-renewal
Plasticity/Heterogeneity

Immunosuppression and MSC
Immunosuppression vs immunoprivilege
Inflammatory context
Specificity (target, MSC subset)

Clinical-grade MSC production:
Processes? Controls?

MSC subsets with different functions?
MSC plasticity?

Native MSC
Localization (between tissues, within tissues)
Roles (immunoregulation?)
Progeny

MSC and tumor stroma
Homing to tumor site
Direct supportive functions
Angiogenesis
Immunosuppression
Malignant niche