

Mesenchymal Stem Cell Applications in the Clinics



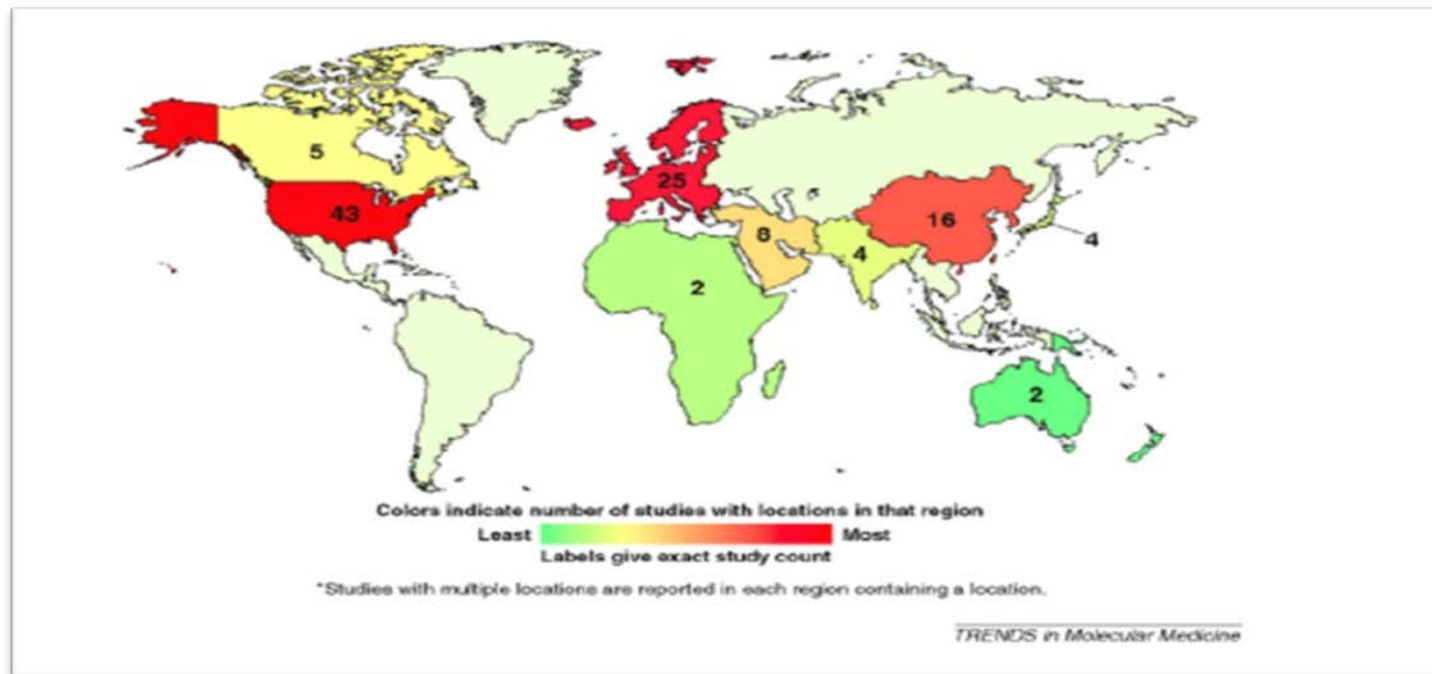
"From Bench to Bedside" 13-17 May 2013 Kayseri

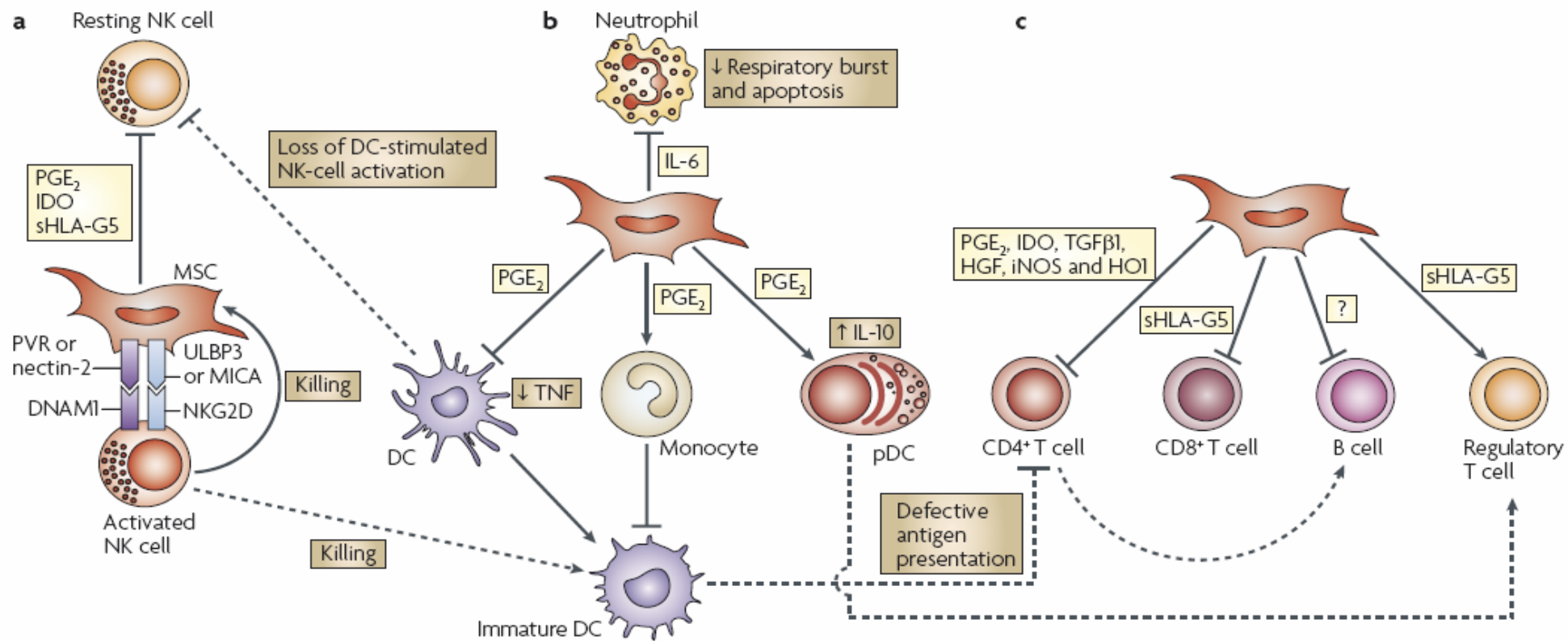
Dr. Mustafa ÇETİN

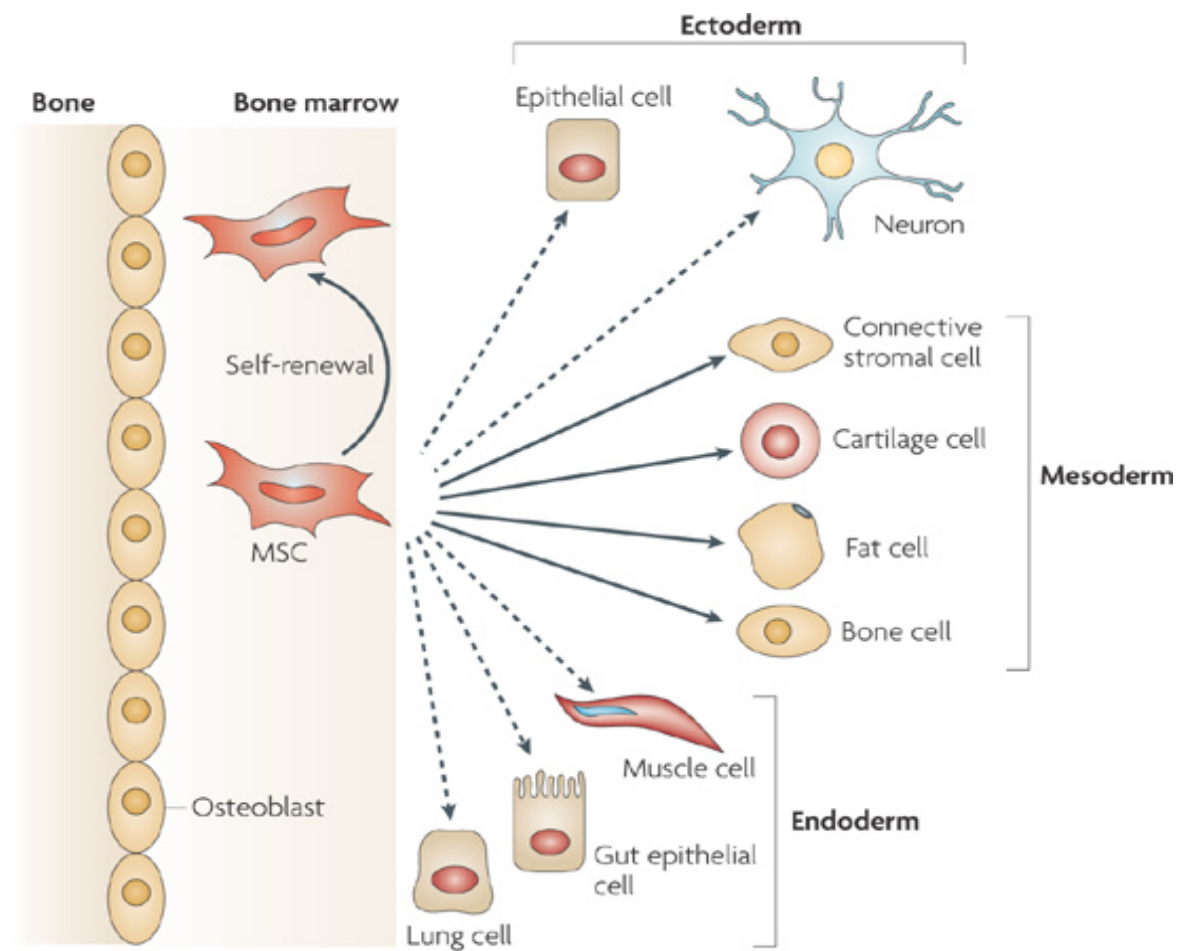


Translational Progress in the Adherent Stem Cell Space

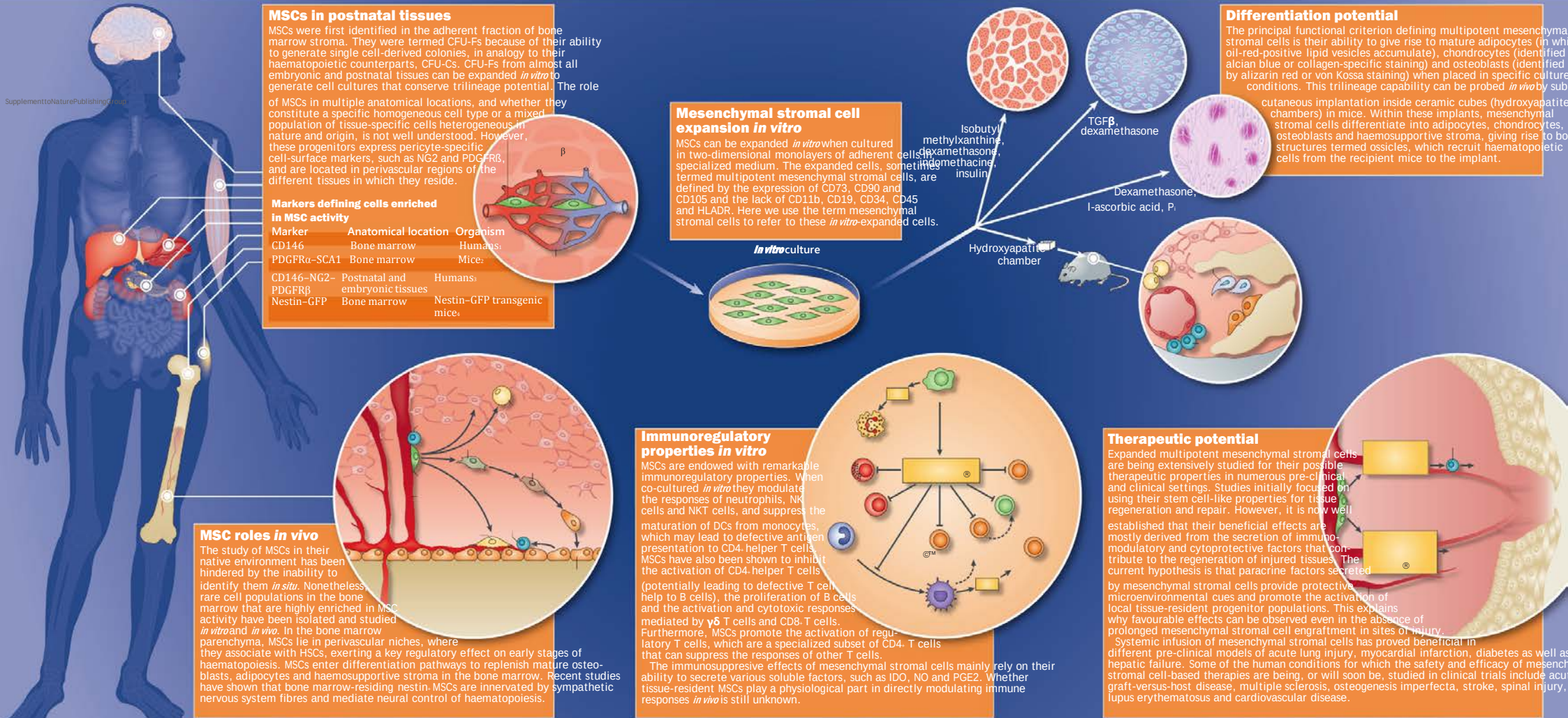
- Till 2010, Over 5400 patients have received adherent stem cell (MSC) treatment, in over 100 trials in over 14 indications
- Since 2011, more than 200 clinical trials have been conducted to test the feasibility and efficacy of MSCs therapy







MSCs are self-renewing, multipotent precursors. They were originally found to reside in the stromal adherent fraction of the bone marrow, where they sustain the homeostatic turnover of non-haematopoietic stromal cells, regulate HSC maintenance and might contribute to vascular stability. The physiological roles of MSCs in anatomical locations other than the bone marrow remain largely undefined. MSCs can be expanded *in vitro* to generate mesenchymal stromal cell cultures, which, under appropriate conditions, can differentiate into adipocytes, chondrocytes and osteoblasts. In more recent studies multipotent mesenchymal stromal cell cultures have been derived from perivascular stem cells expressing pericyte markers in many postnatal tissues. The differentiation capabilities, extraordinary paracrine potential and ease of isolation of *in vitro*-expanded mesenchymal stromal cells have attracted great interest into, and efforts towards, the exploitation of MSCs and their expanded progeny as therapeutic agents for tissue regeneration and repair.



STEMCELL Technologies is committed to providing high quality, standardized media and reagents for your mesenchymal stem cell research. Products are supplied with detailed technical manuals to guide researchers through the procedures. STEMCELL's knowledgeable technical support team is also available to answer any questions and to provide assistance with use of all products.

Isolation: Due to the low frequency at which MSCs occur in specific tissues, it may be desirable to isolate MSCs from a mixed cell population with one of the following kits:

- RosetteSep™ Human MSC Enrichment Kit** (Catalog #15128/15168): for the fast and easy isolation of untouched MSCs from unprocessed human bone marrow.
- EasySep™ Human CD271 Positive Selection Kit** (Catalog #13659): for the isolation of CD271⁺ MSCs with high purity and recovery from human bone marrow.

- EasySep™ Mouse MPC Enrichment Kit for Compact Bone** (Catalog #19711): for the fast and easy isolation of untouched MSCs from mouse compact bone.
- Expansion:** To obtain sufficient numbers of MSCs for basic and translational research, MSCs must be expanded *in vitro*.
- Mesenchymal Stem Cell Culture Kit** (Catalog #05429): xeno-free, serum-free culture kit for *in vitro* expansion of human MSCs. Cells cultured in MesenCult™-XF expand faster, demonstrate superior chondrogenic differentiation potential and more robustly suppress T cell proliferation than cells cultured in serum-based medium.
- Mesenchymal Stem Cell Proliferation Kits** (Human: Catalog #05411; Mouse: Catalog #05511): species-specific serum containing formulations that are optimized for cell expansion and contain prescreened components which minimize lot-to-lot variability.

Colony Assays: All MesenCult™ media products are optimized for performing the colony-forming unit-fibroblast (CFU-F) assay to quantify MSCs.

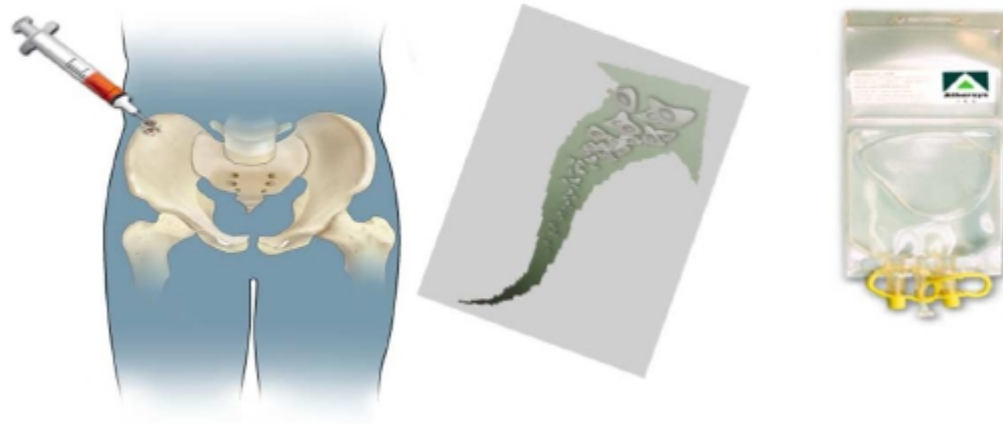
Differentiation: Differentiate human and mouse MSCs to adipocytes or osteogenic progenitors with our optimized MesenCult™ differentiation reagents.

Detection: Aldehyde dehydrogenase has been found to be highly expressed in MSCs.

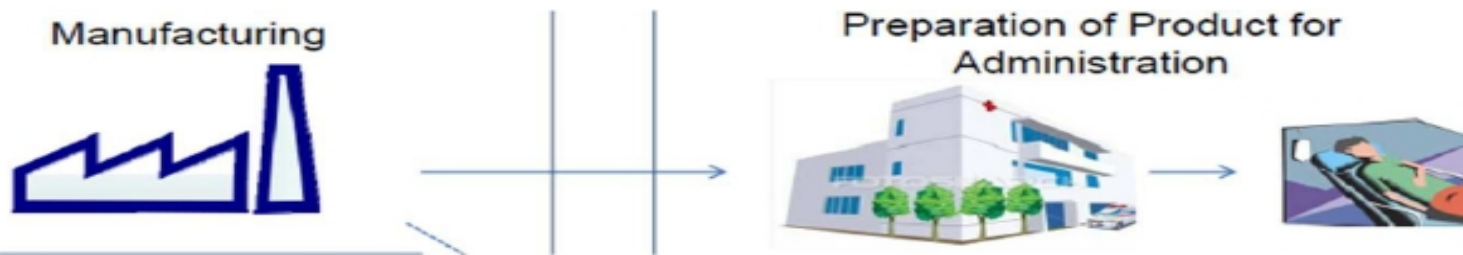
- ALDEFLUOR™** (Catalog #01700): detection of viable stem and progenitor cells based on aldehyde dehydrogenase (ALDH) enzyme activity. Over 150 publications have used it to detect viable stem and progenitor cells of various lineages, including MSCs.

For more information on how STEMCELL Technologies can help your MSC research, please visit our website: www.stemcell.com

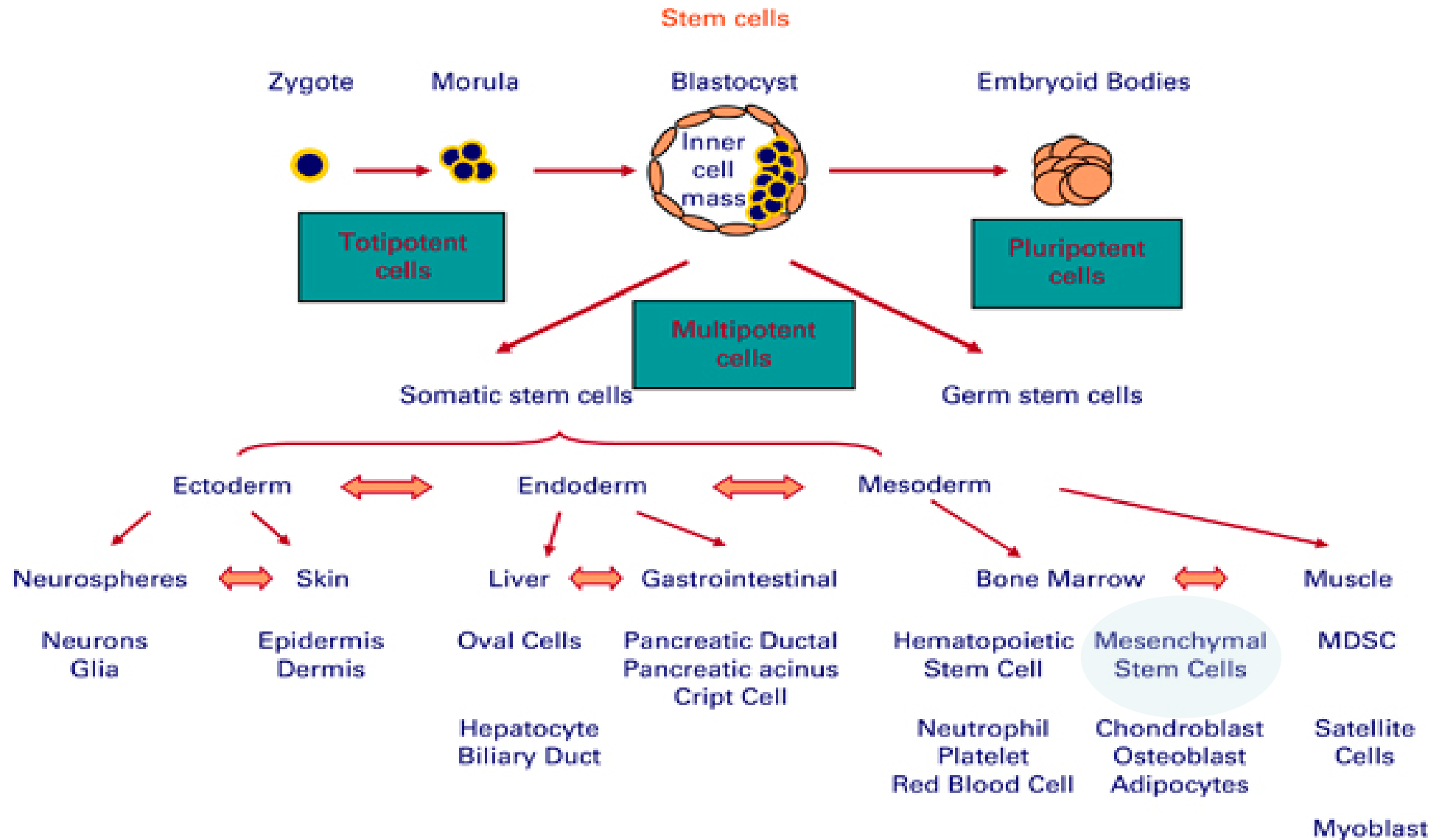
Clinical Development in MSC. Phase I, II, III



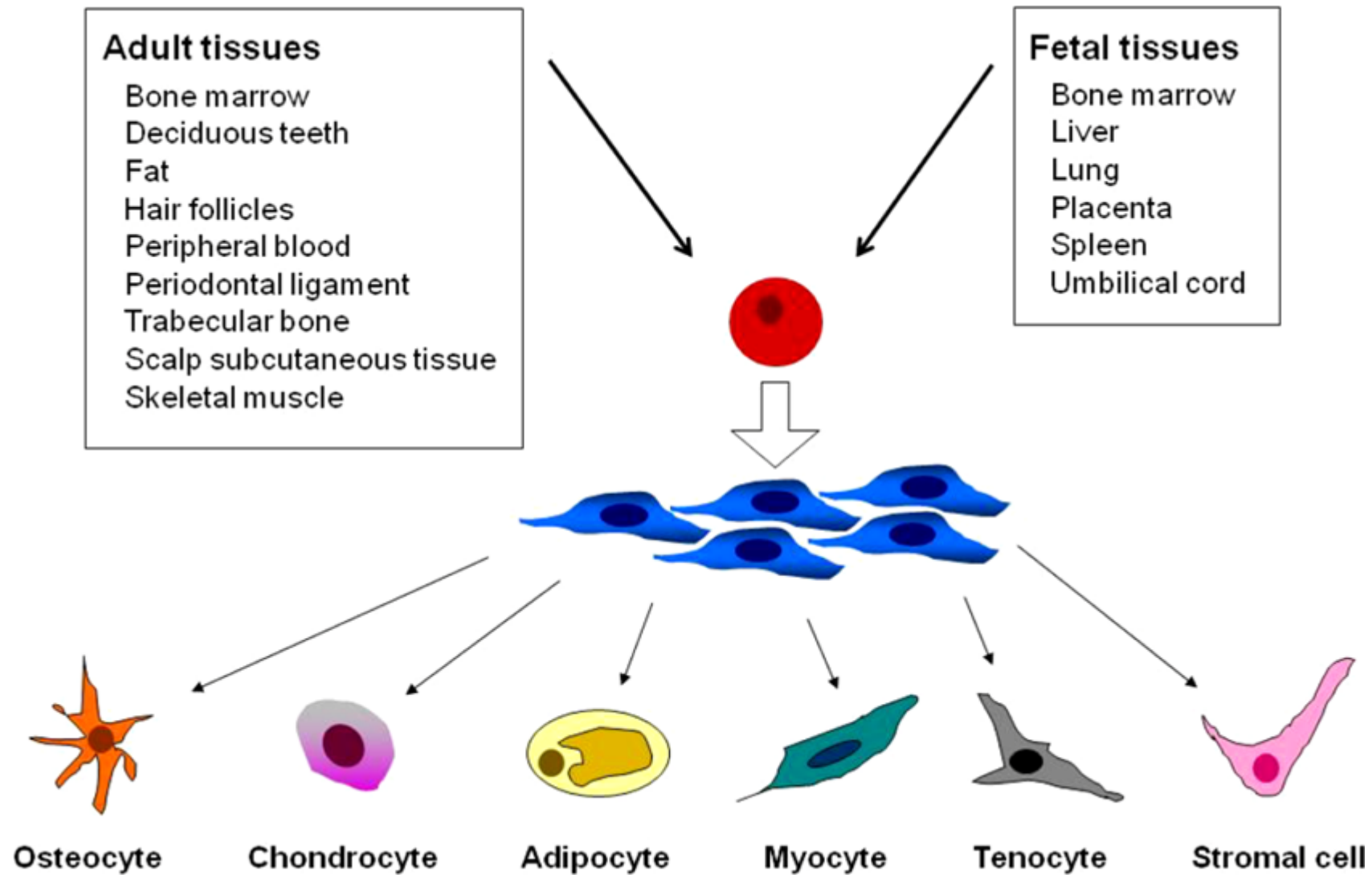
- **Global issues** towards adherent stem cell therapy
(Commercialization moves to medical centers without enough experience)
- Solution –
Good manufacturing Product-quality-vitality-purity-marketing-labeling-delivery
Good Clinical Practice –indications- capacity-(potency) - toxicity-and-survival
Phase Studies: randomized controlled



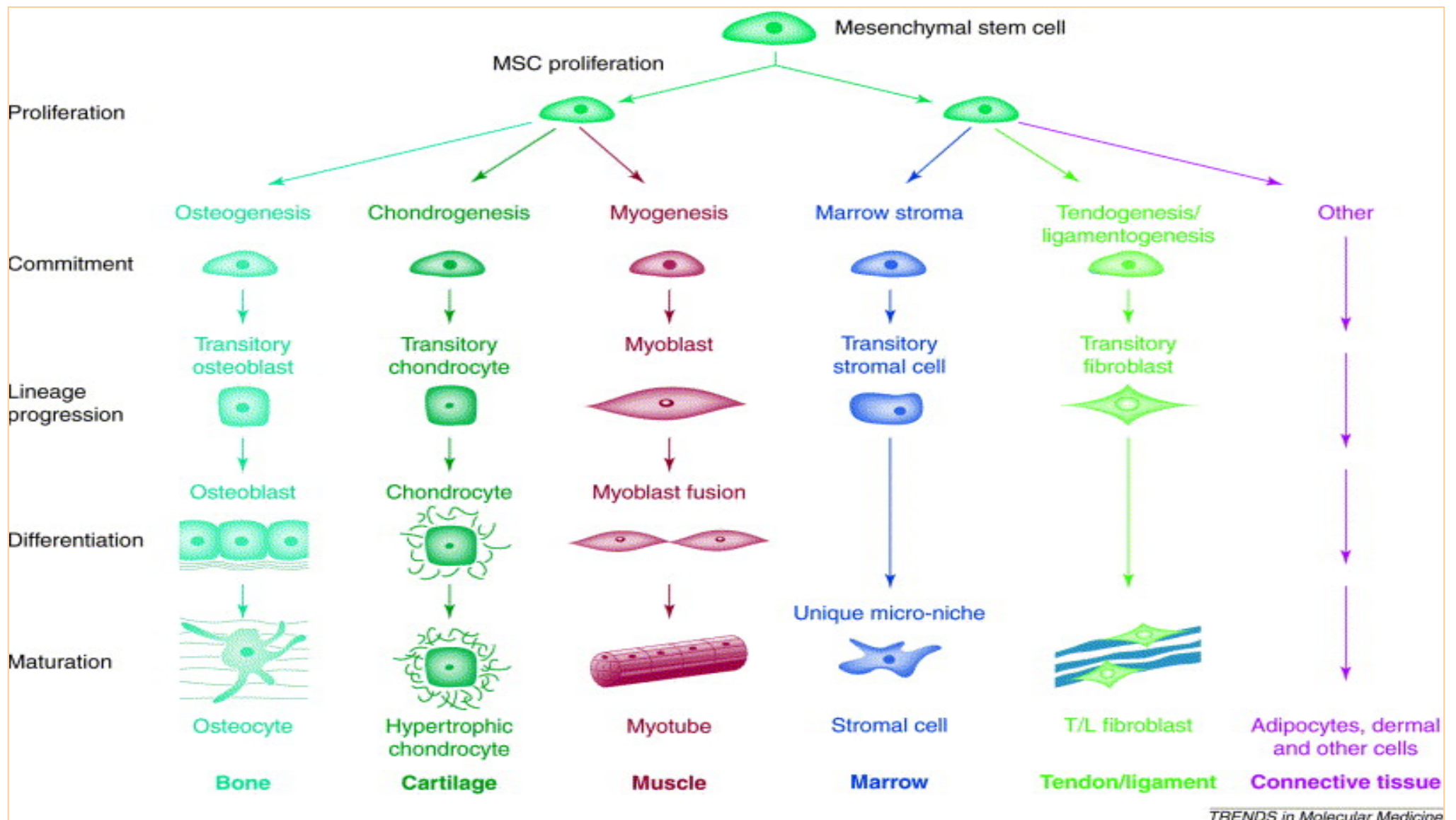
Human Stem Cells



Mesenchymal Stem Cells



Mesenchymal Stem Cells



- Easy isolation, high expansion, reproducible

According to the International Society for Cellular Therapy, human MSCs under standard culture conditions must satisfy at least three criteria:

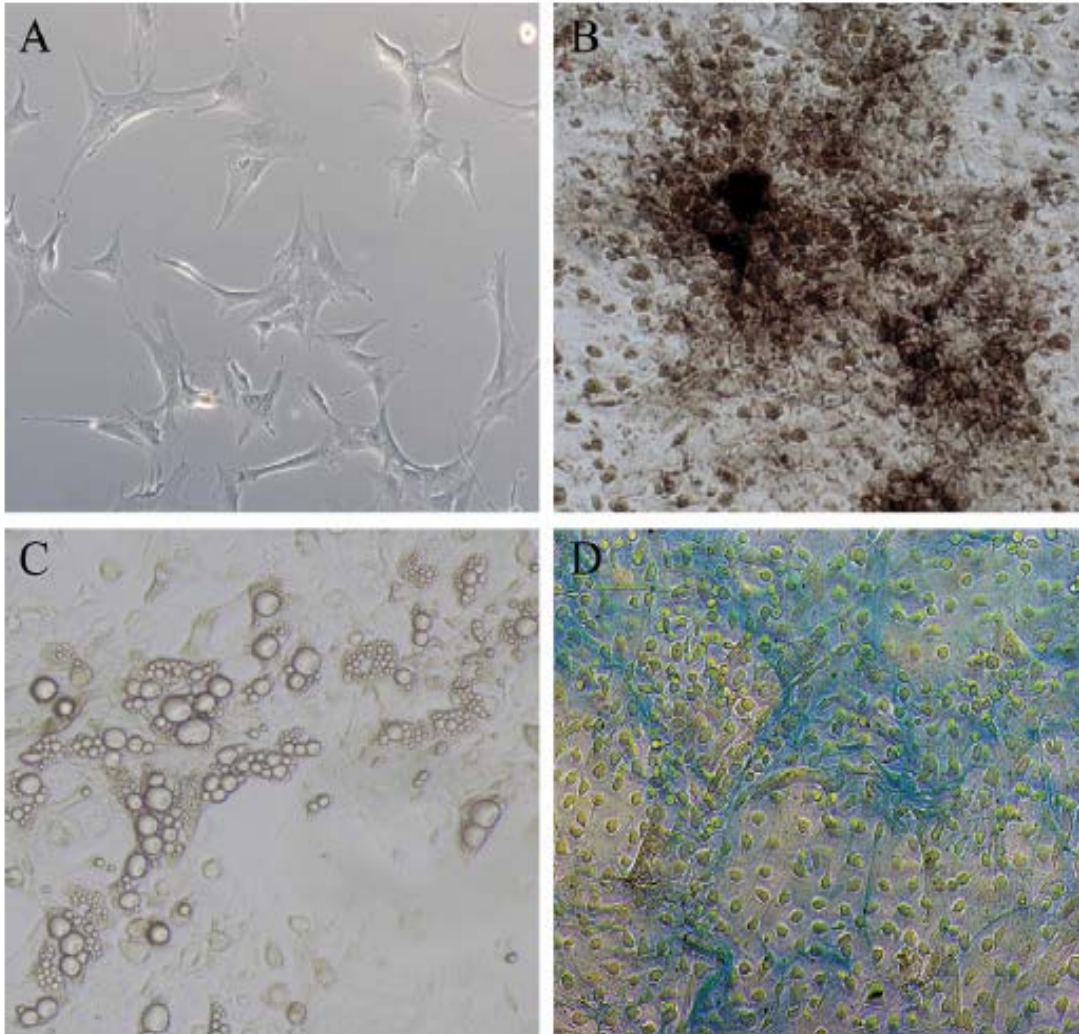
(1) They must be plastic-adherent;

(2) They must express **C105**, **CD73** and **CD90** and not CD45, CD34, CD14, CD11b, CD79 or CD19 and HLA-DR surface molecules by flow cytometry;

(3) They must be capable of differentiating into osteoblasts, adipocytes and chondroblasts

- Other markers that are generally accepted include CD44, CD71, Stro-1, and adhesion molecules such as CD 106, CD166, and CD29

Cultured Mesenchymal Stem Cells; could readily adhere to culture dishes, form fibroblast-like colonies, and are capable of differentiating into adipogenic, chondrogenic, and osteogenic lineages.



- (A) Undifferentiated MSCs grown in monolayer culture
- (B) Cells expressed **ALP** at the 21st day in osteogenic medium.
- (C) **Lipid droplets** were detectable after 2 weeks' inducing.
- (D) Chondrocytes that were detected by **alcian blue** staining, which stains matrix secreted by these cells.

Biological Characteristics of Mesenchymal Stem Cells

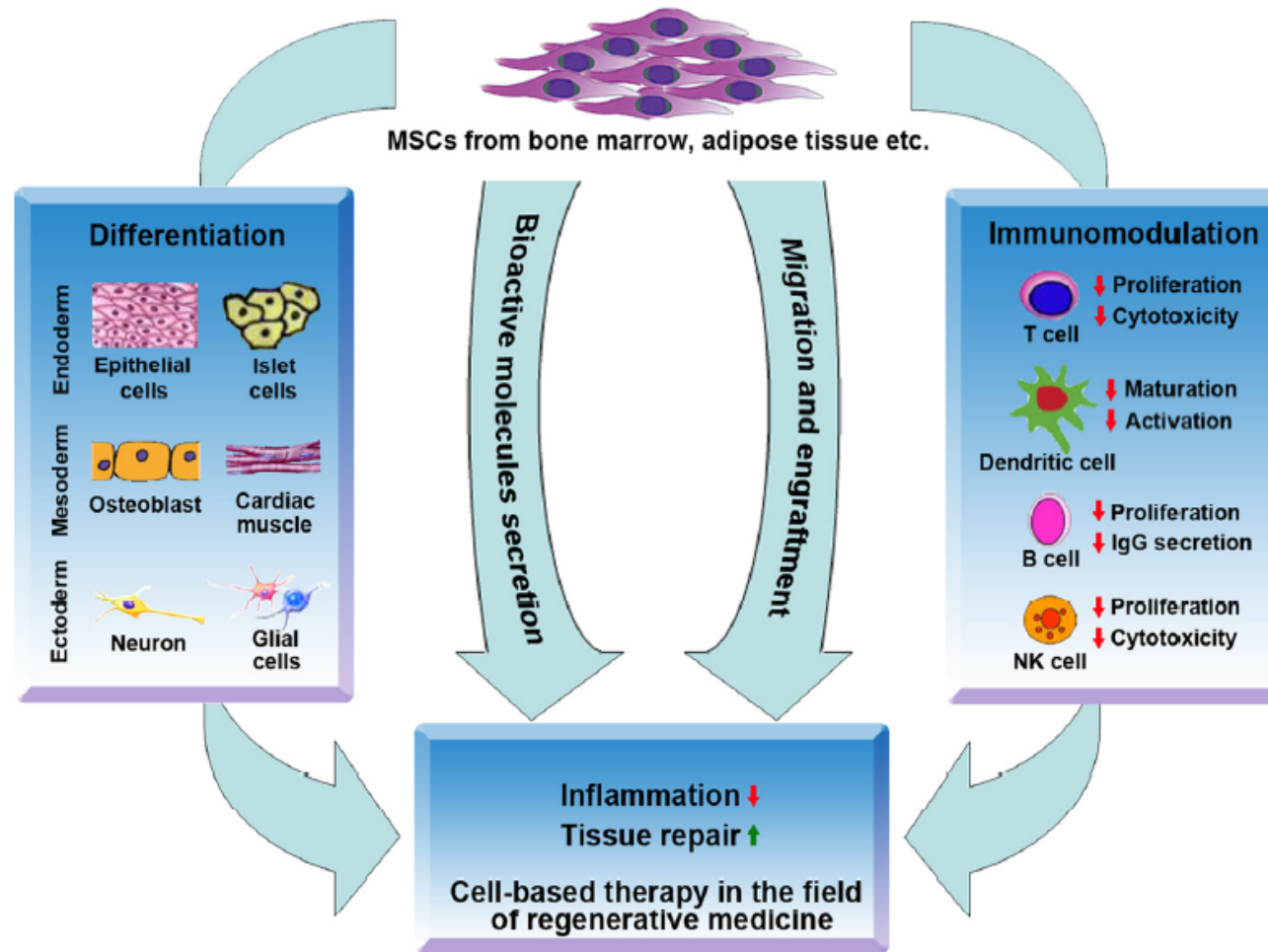


Figure 3 A schematic model demonstrating the biological properties of MSCs that are associated with their therapeutic effects.

Their biological characteristics that contribute to the therapeutic effects...

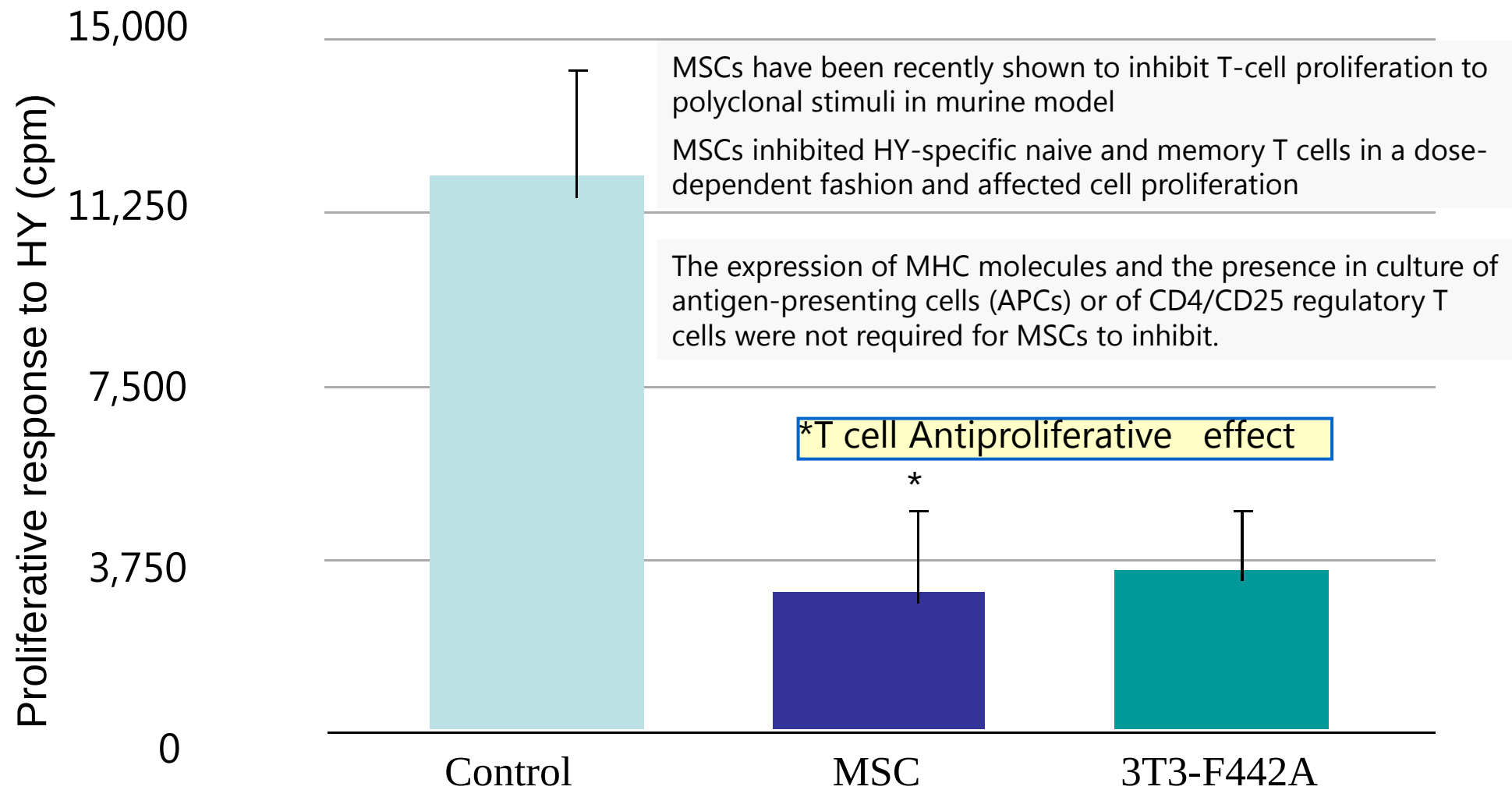
Outline

Mesenchymal Stem Cells

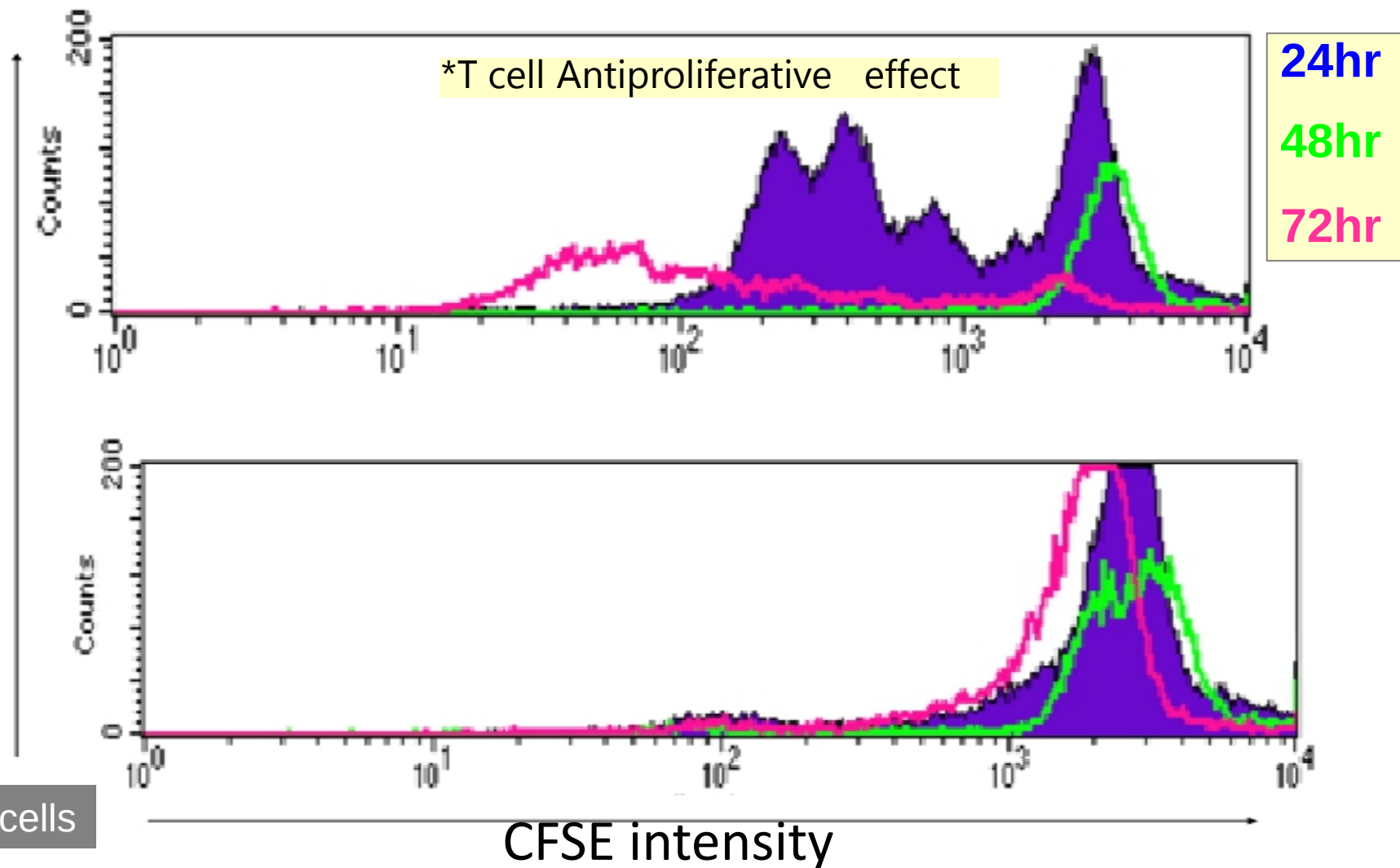
- What type and mechanisms of immunosuppression?
- Bioactive molecules secreted by MSC's
- Tissue repair and niche activity,
- Differentiation of the MSC to other cells
- Conditions for therapeutic applications

CLINICAL APPLICATIONS: PRE-CLINICAL MODELS

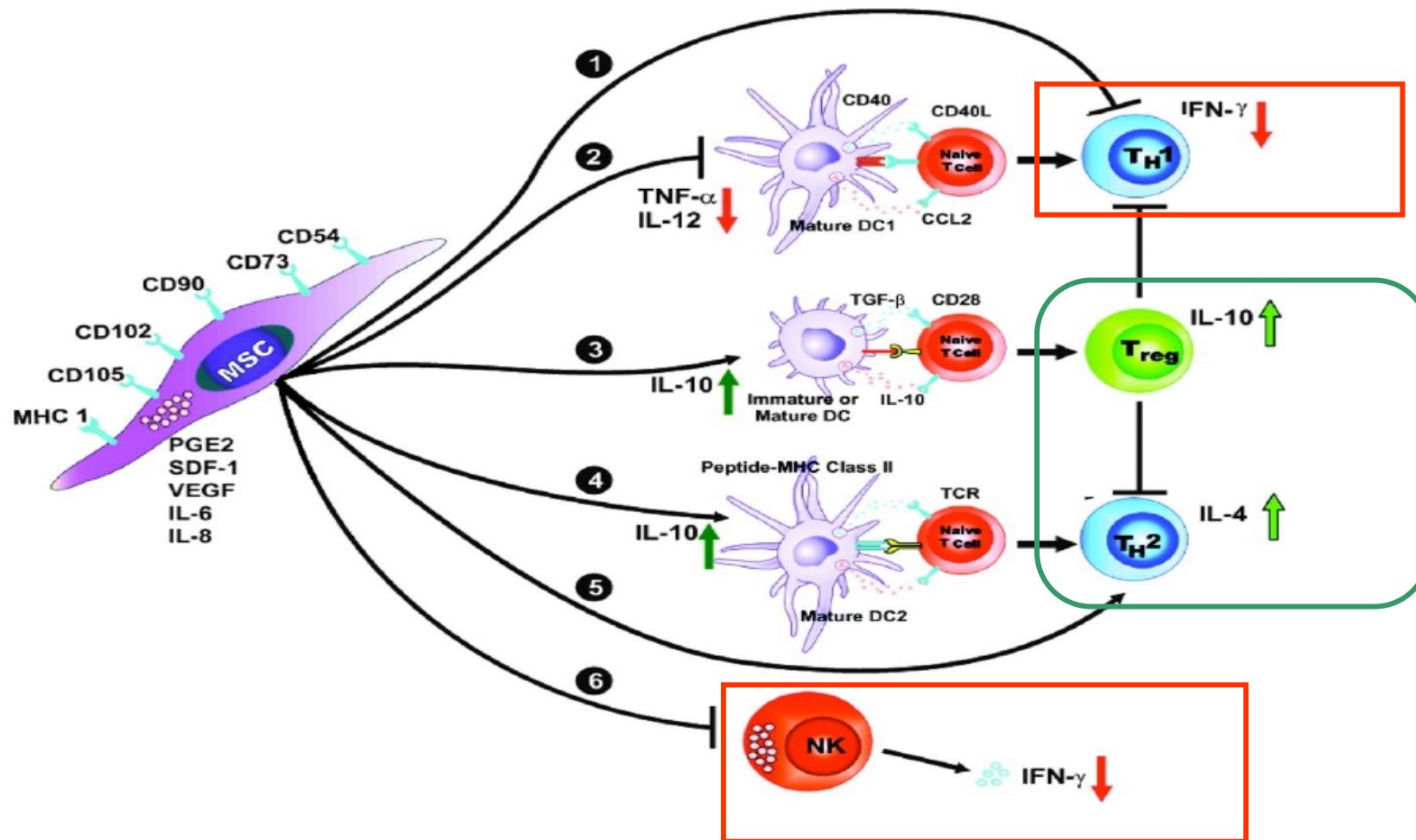
T CELL inhibitory effect of MSC



MSC completely inhibit T cell division



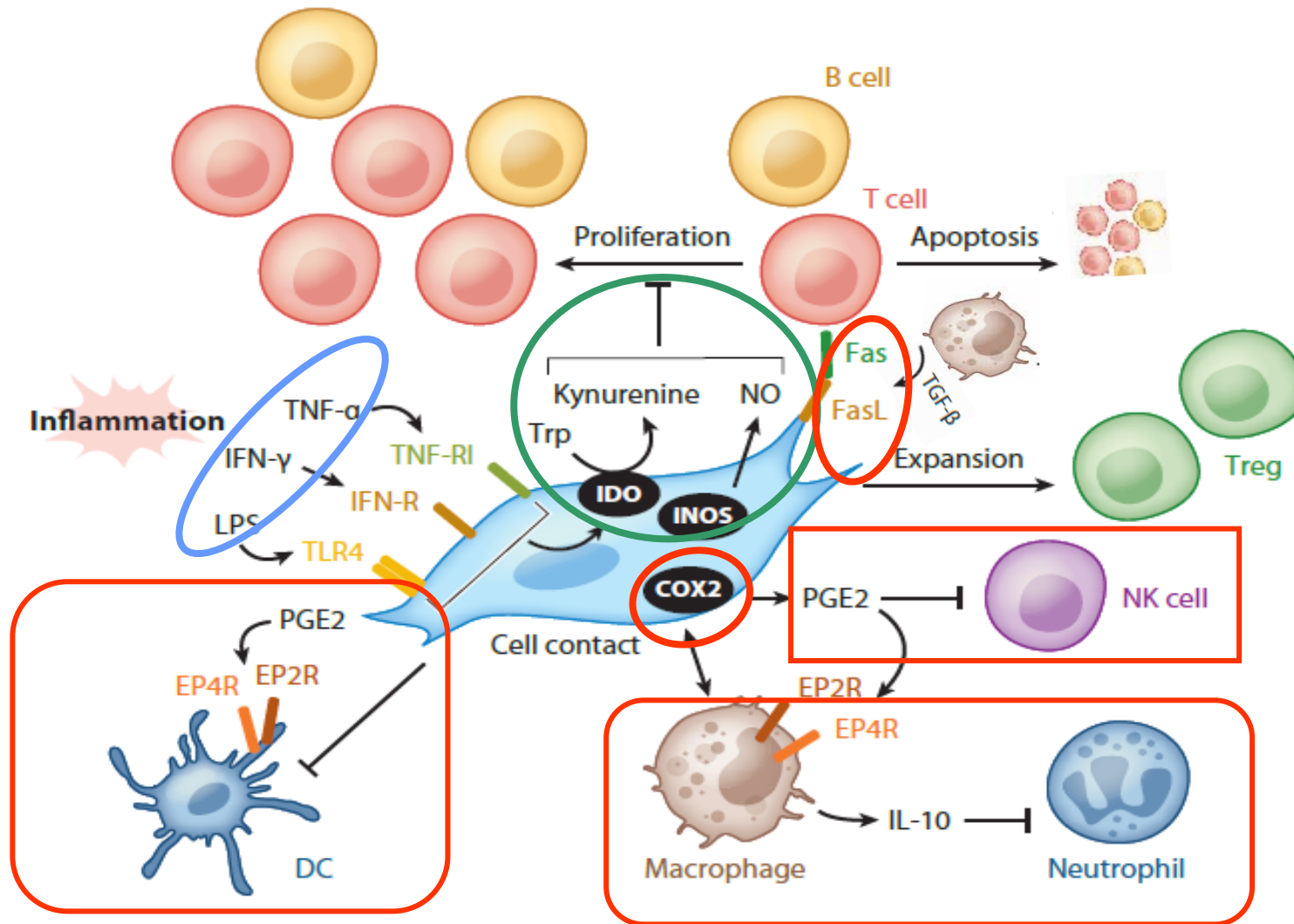
MSC's and APC interactions



hMSCs promote Th2 responses by inhibiting IFN- γ and TNF- α and increasing IL-10.

Also hMSCs alter antigen-presenting cell maturation and induce T-cell unresponsiveness

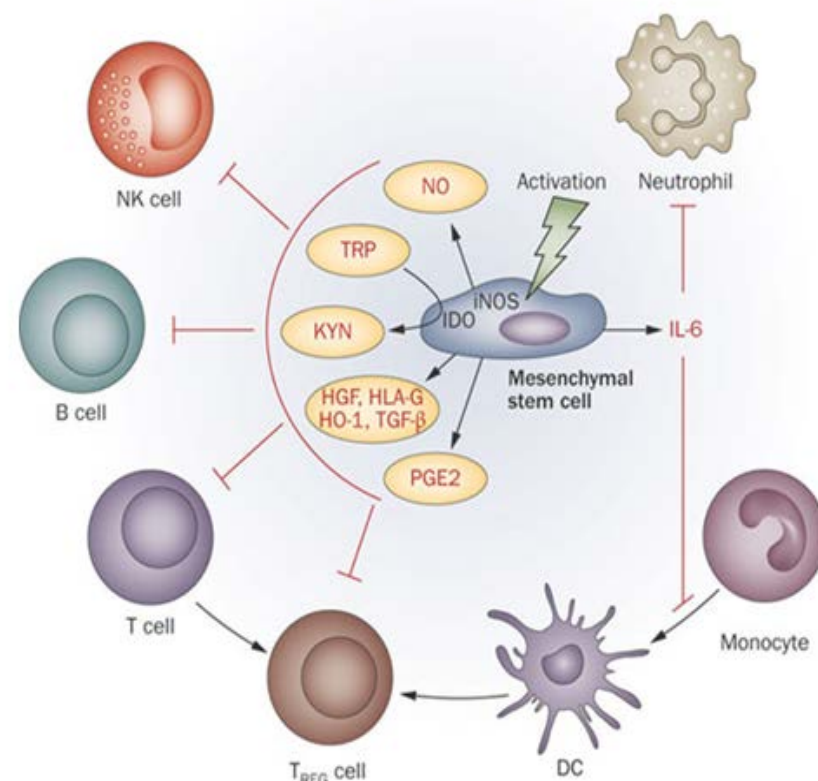
Schematic illustration of the interactions between MSC's and cells of the immune system



Schematic illustration of the interactions between MSC's and cells of the immune system

Immunomodulatory effects of MSCs on immune cells

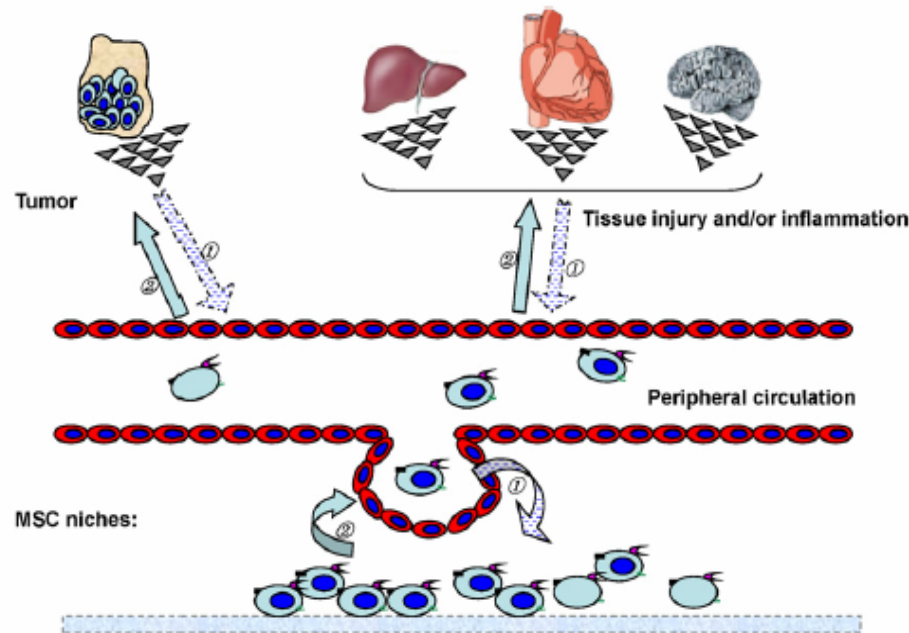
Immune cell type	MSCs' effects
T lymphocyte	Suppress T cell proliferation induced by cellular or nonspecific mitogenic stimuli [44] Alter the cytokine secretion profile of naive and effector T cells [56] Promote the expansion and function of Treg cells [57]
B lymphocyte	Inhibit proliferation of B lymphocyte [58] Affect the chemotactic properties of B cells [59] Suppress B-cell terminal differentiation [60]
NK cell	Alter the phenotype of NK cells and suppress proliferation, cytokine secretion, and cyto-toxicity against HLA-class I- expressing targets [61,62]
Dendritic cells (DCs)	Influence differentiation, maturation and function of monocyte-derived dendritic cells [63] Suppress dendritic cell migration, maturation and antigen presentation [64] Induce mature DCs into a novel Jagged-2-dependent regulatory DC population [65]



MSCs show a strong propensity to ameliorate tissue damage in response to injury and disease.



MSCs to secrete soluble factors that alter the tissue microenvironment functionally outweighs their trans-differentiation ability in affecting tissue repair.



- Hepatocyte growth factor,
- Transforming growth factor-1,
- IL-1, IL-3, IL-6, IL-7, IL-11,
- Stem cell factor,
- FMS like tyrosine kinase 3 ligand,

These factors may enhance regeneration ability of injured tissues, stimulate proliferation and differentiation of endogenous stem-like progenitors found in most tissues, decrease inflammatory and immune reactions (Baddoo et al., 2003).

Conclusions (I)

MSC's exhibit;

- Potent immunosuppressive properties
- Anti-proliferative effect
- Anti-inflammatory effect

Manage "Immunomodulatory effects" and "Stem cell niche activity – Regenerative Medicine" and "Cytoprotective & tissue repair activity"

CLINICAL APPLICATIONS: PRE-CLINICAL MODELS

"Immunomodulating
activity"

"Tissue Protection"

"Regenerative
Medicine"

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"Immunomodulating
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GvHD

Graft rejection

Autoimmune diseases

Type 1 Diabetes

Crohn's DS. ,Colitis

CLINICAL APPLICATIONS: PRE-CLINICAL MODELS

"Immunomodulating
activity"

GvHD

Graft rejection

Autoimmune diseases

Type 1 Diabetes

Crohn's DS. ,Colitis

"Tissue Protection"

Pulmonary fibrosis

Myocardial infarction

Renal ischaemia

Tissue repair

"Regenerative
Medicine"

CLINICAL APPLICATIONS: PRE-CLINICAL MODELS

"Immunomodulating
activity"

GvHD
Graft rejection
Autoimmune diseases
Type 1 Diabetes
Colitis

"Tissue Protection"

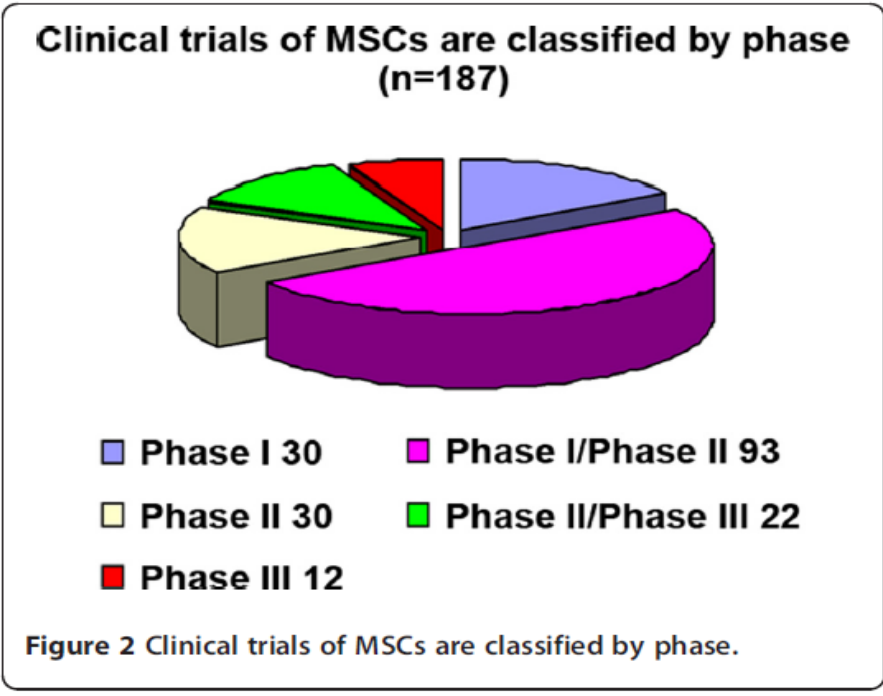
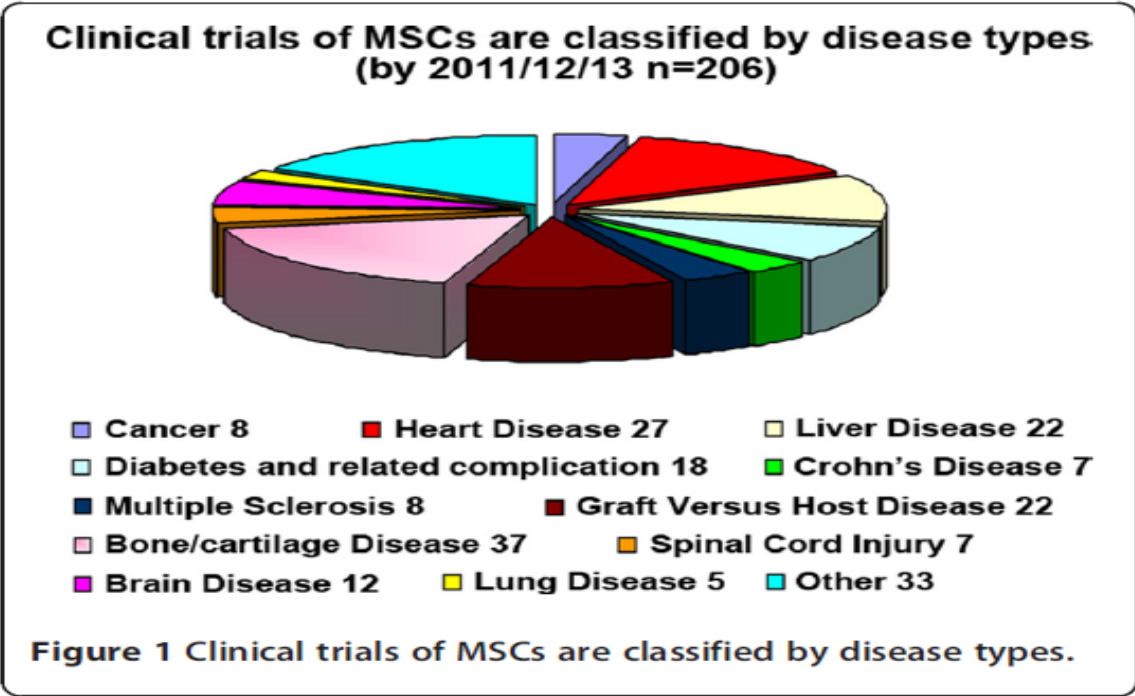
Pulmonary fibrosis
Myocardial infarction
Renal ischaemia
Liver cirrhosis

"Regenerative
Medicine"

Bone & cartilage repair
Haemopoietic recovery
Type 2 Diabetes

Clinical applications of mesenchymal stem cells

Shihua Wang, Xuebin Qu and Robert Chunhua Zhao*



Clinical applications of mesenchymal stem cells

Journal of Hematology & Oncology 2012, **5**:19



JOURNAL OF HEMATOLOGY
& ONCOLOGY

Indication	Number of studies
Immunomodulation	48
Multiple sclerosis/atherosclerosis	12
Type 1 diabetes	12
Crohn's disease	10
Systemic lupus erythematosus/colitis	4
Rheumatoid arthritis/Sjögren's syndrome	3
Buerger's disease/sickle cell disease	2
HIV	1
Limbus corneae insufficiency syndrome	1
Periodontitis	1
Progressive hemifacial atrophy	1
Retinitis pigmentosa	1
Tissue protection	76
Myocardial infarction/stroke/ischemia	34
Liver cirrhosis	20
Alzheimer's/Parkinson's disease	4
Amyotrophic lateral sclerosis	4
Fibrosis/emphysema	4
Necrosis	4
Acute kidney injury	2
Bronchopulmonary dysplasia	2
Multiple system atrophy/multiple trauma	2

Indication	Number of studies
Regenerative medicine	69
Osteoarthritis/osteogenesis imperfecta	22
Bone/cartilage repair	18
Spinal cord injury/neuroblastoma	8
Anemia	4
Type 2 diabetes	4
Dilated cardiomyopathy	4
Wound healing/umbilical cord varices	3
Ataxia	2
Autism	1
Epidermolysis bullosa	1
Erectile dysfunction	1
Wilson's disease	1
Graft enhancement	27
GvHD	23
Hematopoietic malignancies	4

Table 1 Clinical trials (registered as of June 2, 2012) using mesenchymal stem cells

Graft-versus-host disease

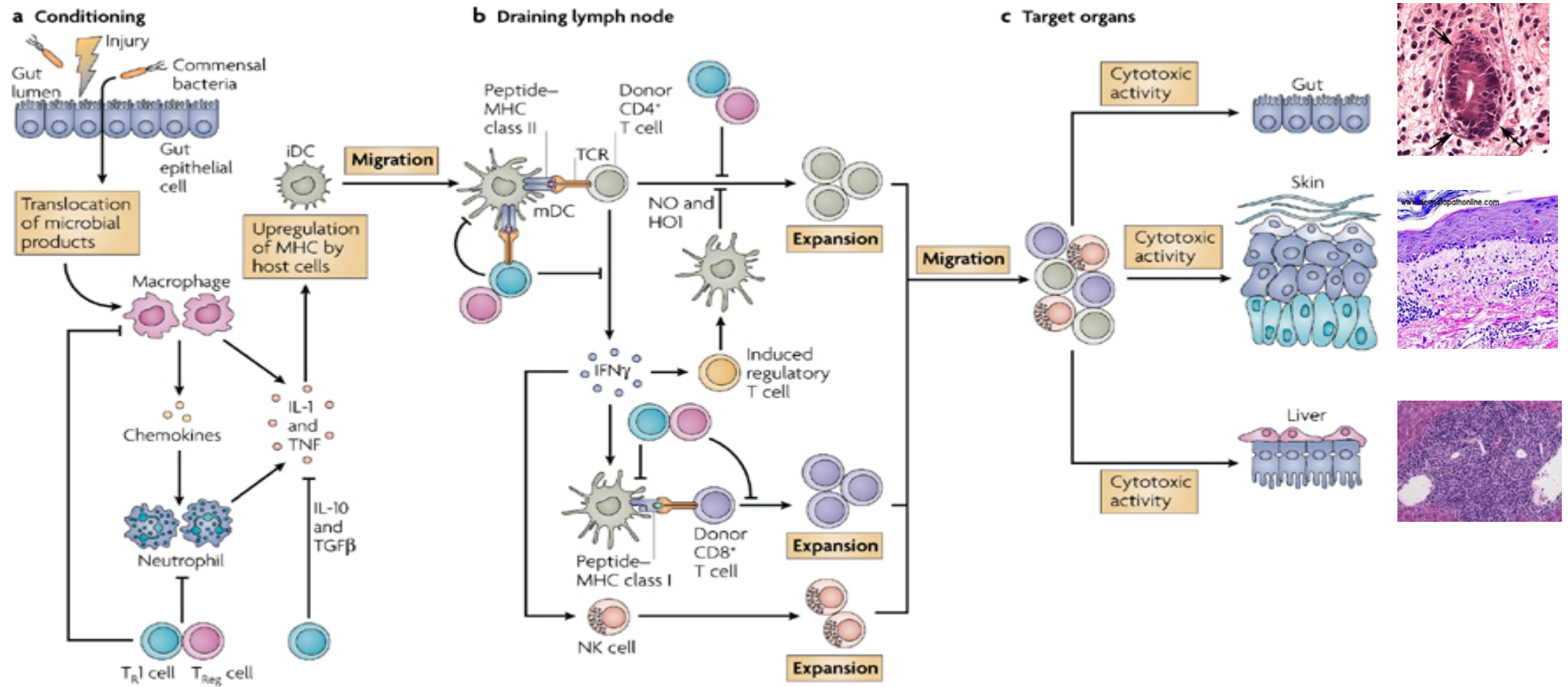
GVHD

GVHD occurs after allogeneic hematopoietic stem cell transplant and is associated with high morbidity and mortality.

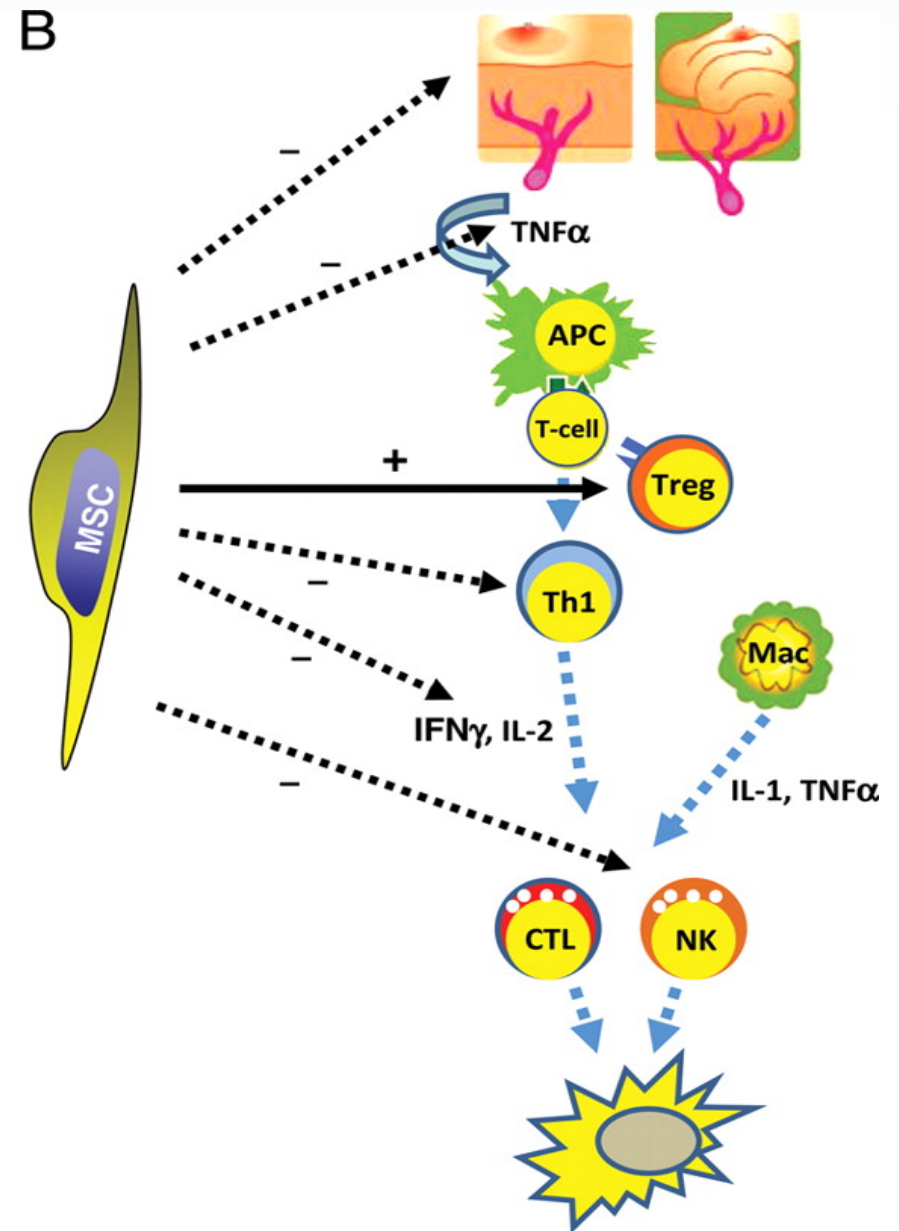
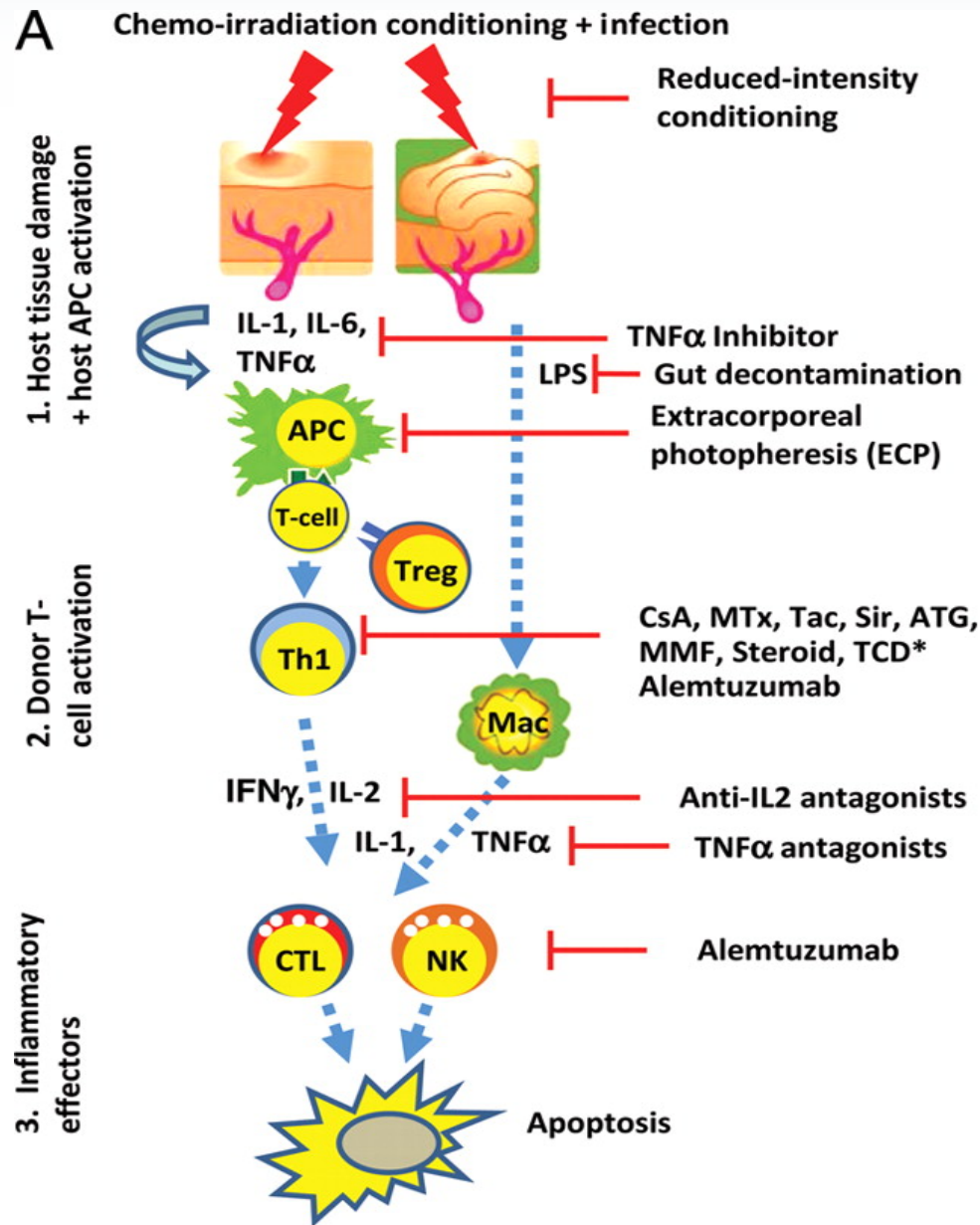
Currently, corticosteroids are the gold standard for initial treatment of aGVHD. However, they are only effective for some patients



GVHD



GVHD



MSC therapies have been most extensively studied in steroid-refractory GvHD

- The first case of successful treatment of severe refractory acute GvHD of the gut and liver in a pediatric patient using ex vivo expanded haplo-identical human MSC was reported by Le Blanc.
- While a prompt amelioration of GvHD was observed after the administration of MSC, symptoms recurred. However, these symptoms were responsive to a second administration of MSC

GVHD

Compassionate use: Now(5/2006) APPROVED for PAYMENT & USE

- 12 pediatric patients [5months to 15 years of age]
- Suffering from treatment resistant GVHD
- Prochymal (MSC) infusions (3-21

(12/12) showed a CLINICAL RESPONSE

(7/12) , 58% achieving COMPLETE RESPONSE.

- No infusion toxicity or adverse events.

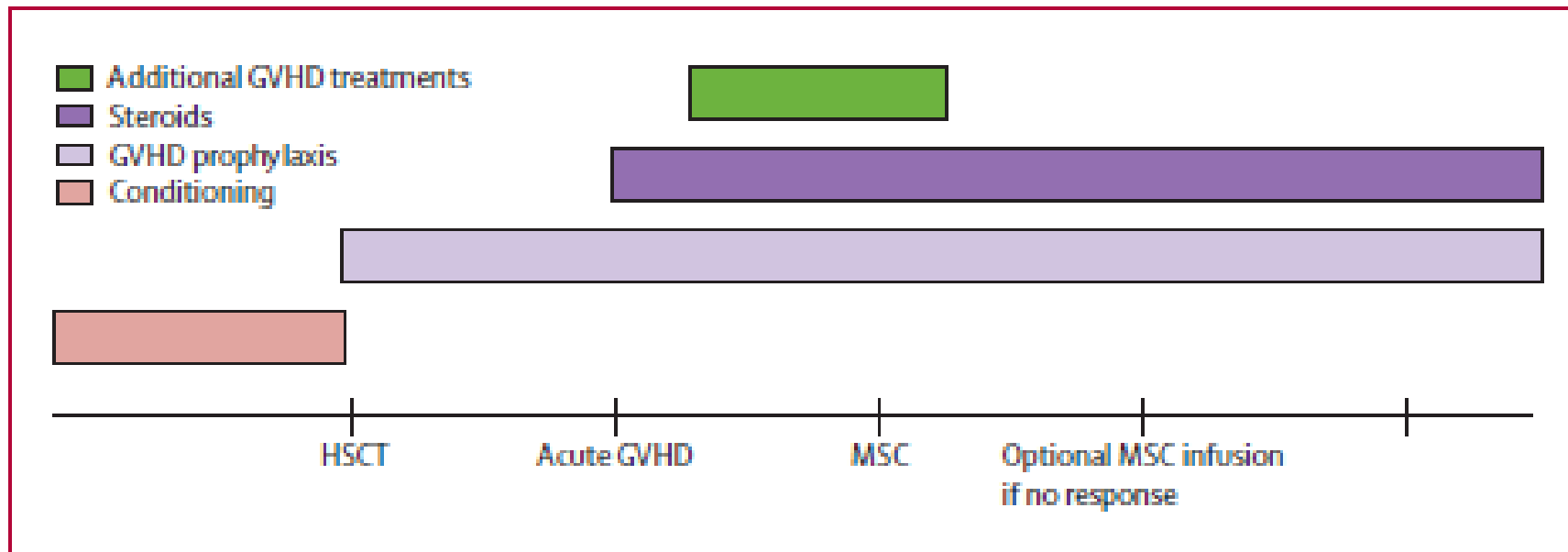
MSC increase survival of aGVHD patients

The clinical response was striking, with improvement of liver and intestinal function.



Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study

Katarina Le Blanc, Francesco Frasson^{*}, Lynne Ball^{*}, Franco Locatelli, Helene Roelofs, Ian Lewis, Edoardo Lanino, Berit Sundberg,



- A later phase II clinical study (25 children and 30 adults)
- 55 steroid-resistant patients with severe acute disease

MSC increase survival of aGVHD patients

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Mesenchymal Stem Cell Expansion EBMT Consortium,

- 55 patients with acute GVHD grade 2–4.
- Transfer of a median 1.4×10^6 cells/kg-bw

Complete responses in 55%

Partial or complete responses in 71%

Cells were expanded ex vivo and generated from either HLA-identical siblings, haploidentical donors, or third-party mismatched donors.

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

*At last data collection, March, 2007.

Table 4: GVHD response and outcome

MSC increase survival of aGVHD patients

The clinical response was striking, with improvement of liver and intestinal function.



Measure	
Donors	
Number of donors	45
Donor sex (male/female)	25/20
Donor age	36 (1-67)
Number of infusions by donor type	
HLA- identical sibling	5
HLA- haploidentical donor	18
Unrelated HLA- mismatched donor	69
Volume of bone marrow harvested (mL)	60 (32-220)
Median MSC cell dose (x10 ⁶ /kg, range)	1.4 (0.4-9)
Culture passage at MSC harvest	
Passage 1	14
Passage 2, 2+3	42, 7
Passage 3, 3+4	23, 2
Passage 4	4
Number of MSC infusions	
One	27
Two	22
Three	4
Four	1
Five	1

Data are number or median (min-max range). MSC=mesenchymal stem cell.

Table 3: Mesenchymal-stem-cell donor and graft characteristics

- Most interestingly, there was no difference in the response rates or side-effects between patients receiving mesenchymal stem cells from third-party mismatched donors compared with those in patients receiving cells from HLA-identical siblings or from haploidentical family members
- **MSCs induced a 70% initial response rate that was not related to HLA match.**
- None of the patients had side effects either during or immediately after the MSC infusion

MSC increase survival of aGVHD patients

The clinical response was striking, with improvement of liver and intestinal function.

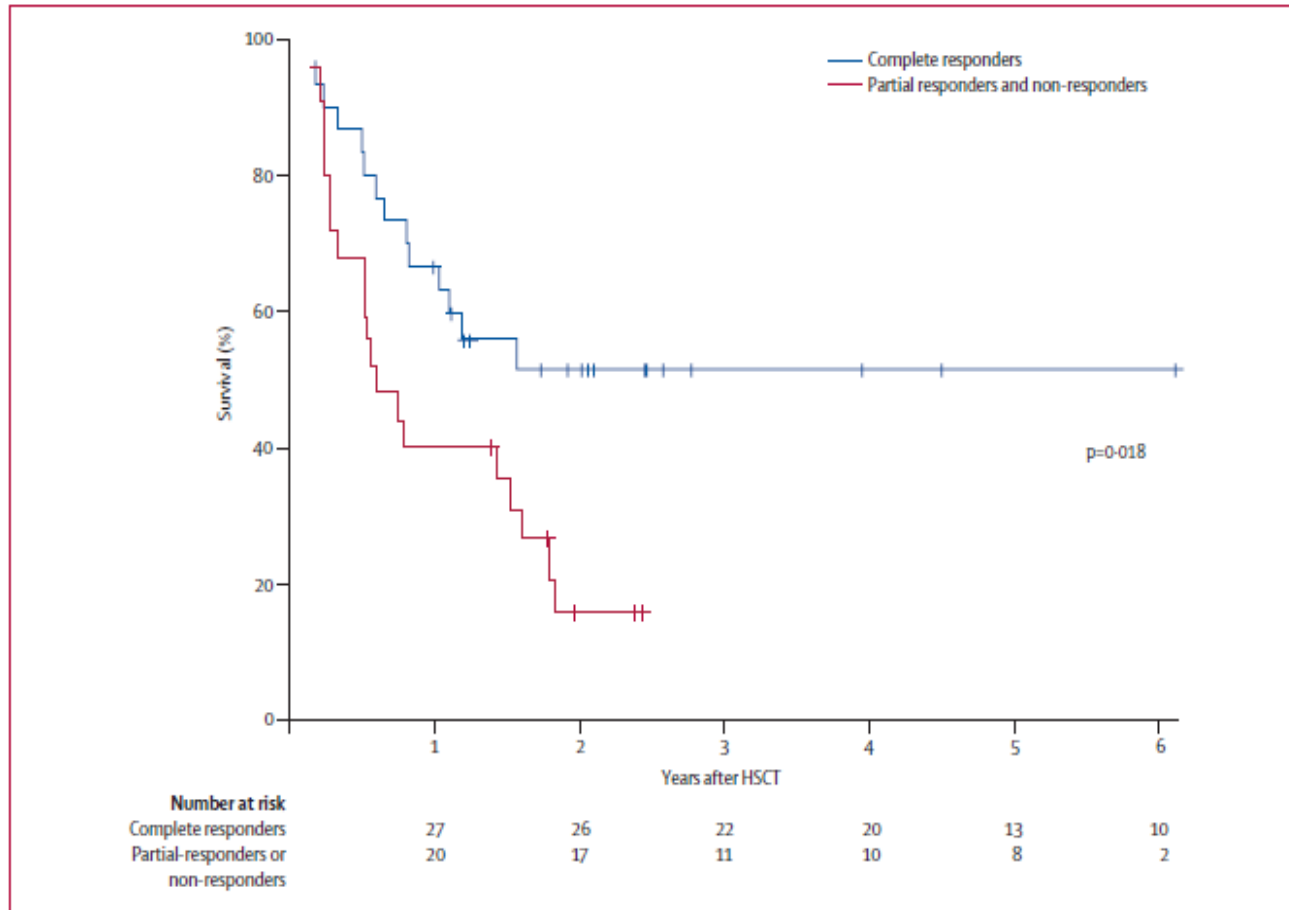


Figure 2: Survival from time of haemopoietic-stem-cell transplantation in patients given mesenchymal stem cells

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.

MSC's derived from bone marrow might be a safe and effective treatment for patients with severe, acute GVHD who do not respond to corticosteroids and other immunosuppressive therapies.

Just over half of patients with a complete response were alive at 2 years.

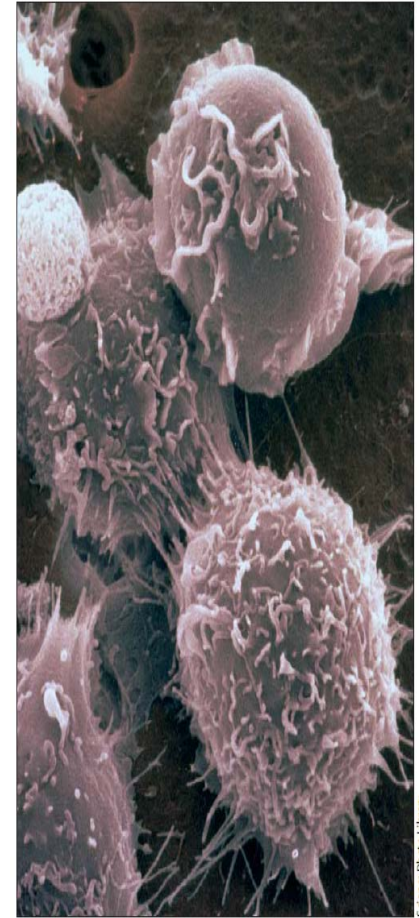
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Mesenchymal stem cells as cellular immunosuppressants

- Dendritic cells (DC) are potent APC for naïve T-cells, and are critical in donor T-cell activation during acute GvHD (Shlomchik, 2007).
- MSC inhibit differentiation of monocytes to DC, and furthermore, affect DC differentiation, activation, and function (Uccelli et al., 2008).
- MSC also inhibit natural killer (NK) cell proliferation and cytokine production, and could potentially modulate DC function through their effects on NK cells (Spaggiari et al., 2006).
- In the light of these effects, MSC might suppress allo-reactivation of donor T-cells against the host in the setting of GvHD



Within the context of innate immunity, MSC alter antigen-presenting cell (APC) development, maturation, and function

MSC as an adjunct to steroid therapy in the treatment of steroid responsive acute GvHD

A randomized, prospective, open label trial at 16 cancer centers in the USA with 32 patients with grades II-IV GVHD. *Now FDA OK PHASE III.*

- 77% complete remission in 28 days.
- 61% at 6 months had a durable response requiring
- No additional immunosuppressive therapy or clinical intervention
- 95% were alive at 6 months compared to patients receiving additional immunosuppression (25% survival).
- No adverse events



Study	N	Age (range)	GvHD organ/grade	MSC source	Passage/media	Dose (M, 10 ⁶ MSC)/schedule	Results
Kebriaei et al. (2009)	32	52 (34–67)	Grade II: 21 Grade III: 8 Grade IV: 3	BM, third party (Prochymal)	5/FBS	2 or 8 M/kg at 1 and 3 days after GvHD + steroids	94% initial response (77% CR, 16% PR), 61% sustained CR; No difference between high/low MSC dose; No infusional toxicity; three disease relapse

Phase II clinical trials of third-party MSC to ameliorate steroid-refractory acute GvHD

(Further to the EBMT MSC trial, a pediatric phase II study of third-party, “off-the-shelf,” mismatched MSC (Prochymal®, Osiris Therapeutics, Inc.) for steroid refractory acute GvHD has also been reported)

Study	N	Age (range)	GvHD organ/grade	MSC source	Passage/media	Dose (M, 10 ⁶ MSC)/schedule	Results
Ringden et al. (2006)	8	56 (8–61)	All GI Grade III: 6 Grade IV: 2	BM, third party/slb/haplo	1–4/FBS	1 M/kg (range 0.7–9); 1 dose, <i>n</i> = 5; 2 dose, <i>n</i> = 3	6/8 CR (1/2 kids); 5/8 OS; no infusional toxicity; one disease relapse
Fang et al. (2007)	6	39 (22–49)	S+L or GI Grade III: 2 Grade IV: 4	Adipose, third party/haplo	5/FBS	1 M/kg MSC; 1 dose, <i>n</i> = 5; 2 dose, <i>n</i> = 1	5/6 CR, 4/6 OS at 40 months; no infusional toxicity; one disease relapse
Le Blanc et al. (2008)	55	22 (0.5–64)	S10, GI 31, L2 Grade II: 5 Grade III: 25 Grade IV: 25	BM, third party/slb/haplo	2 (1–4)/FBS	1.4 M/kg (range 0.4–9); 1 dose (range 1–5)	CR: 68% kids, 43% adults; PR: 16% kids, 17% adults; 2-year OS: 53% for CR vs. 16% others; no infusional toxicity; 3 relapse
Von Bonin et al. (2009)	13	58 (21–69)	All S+L+GI Grade III: 2 Grade IV: 11	BM, third party	1–2/platelet lysate	0.9 M/kg (range 0.6–1.1); 2 doses (range 1–5);	2/13 CR, 5/13 mixed response; 4/13 OS at median 257 days; No infusional toxicity; no relapse
Muller et al. (2008)	2	4, 14	Grade II (S, GI) Grade III (S, L, GI)	BM, haplo/third party	Max 6 weeks culture/FBS	0.4 M/kg, 3 M/kg 1 dose	1 CR, 1 NR with subsequent relapse; no infusional toxicity
Lucchini et al. (2010)	8	10 (4–14)	Grade I: 3, S Grade II: 1, S Grade III: 0 Grade IV: 4, GI	BM, third party	Platelet lysate	1.2 M/kg (range 0.7–2.8); 1 dose	3/8 CR, 2/8 PR, 3/8 NR 5/8 OS; no infusional toxicity; no relapse
Kurtzburg et al. (2009)	59	8	Grade II: 6 Grade III: 20 Grade IV: 33	BM, third party (Prochymal)	5/FBS	2 M/kg; 8 biweekly × 4 weeks, followed by 4 infusions weekly × 4 if PR	64% ORR at day 28; 76 vs. 9% survival at day 100; no infusional toxicity
Martin et al. (2010)	260	44 MSC; 40 control	MSC/control B: 38 vs. 23 C: 88 vs. 50 D: 47 vs. 14	BM, third party (Prochymal)	5/FBS	2 M/kg; 8 biweekly × 4 weeks, followed by 4 infusions wklly × 4 if PR	No diff in durable CR between MSC and control; liver, GI GvHD significantly better response 81 vs. 68%, <i>p</i> = 0.035

MSC as a cellular therapy to ameliorate chronic GvHD

Significant improvements have been reported following MSC therapy in patients with sclerodermal-type chronic GvHD

- Patients with extensive skin changes and ulcers showed significant improvement when treated with four to eight intra-bone marrow injections of MSC at a dose of $1-2 \times 10^7$ MSC/kg.
- The administration of MSC and improvement in chronic GvHD was associated with an increase in the proportion of Th2 lymphocytes and a reduction in the proportion of Th1 lymphocytes.

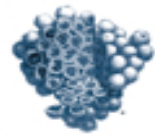
One change noted following MSC administration and possibly associated with the improvement in the symptoms of chronic GvHD was a reversal in the Th1 to Th2 lymphocyte ratio. (Zhou et al., 2010).

Study	N	Age (range)	GvHD organ/grade	MSC source	Passage/media	Dose (M, 10^6 MSC)/schedule	Results
Zhou et al. (2010)	4	42 (38–43)	Extensive, sclerodermal features	BM, third party	3–6/FBS	$1-2 \times 10^7$ MSC/kg; 4–8 intra-BM injections per patient	4/4 significant improvement; no infusional toxicity

MSC; in the treatment of chronic-extensive GvHD

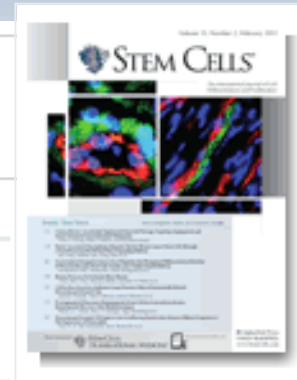
Table 3 | Results of clinical trials utilizing MSC for chronic GvHD.

Study	N	Age (range)	GvHD organ/grade	MSC source	Passage/media	Dose (M, 10 ⁶ MSC)/schedule	Results
Muller et al. (2008)	3	15 (15–17)	Extensive chronic	BM, third party/sib/haplo	Max 6 weeks culture/FBS	2.0 M/kg (range 1.4–3.0); 1 dose, <i>n</i> = 1; 2 dose, <i>n</i> = 2	1/3 improvement; no infusional toxicity; no relapse
Lucchini et al. (2010)	5	9 (5–15)	Chronic skin + mucosa, <i>n</i> = 4; chronic skin + liver, <i>n</i> = 1	BM, third party	expanded in platelet-lysate medium	1.1 M/kg (range 0.7–1.4); 1 dose, <i>n</i> = 4; 2 dose, <i>n</i> = 1	1/5 CR with reflare, 2/5 PR, 2/5 NR; no infusional toxicity; no relapse; <i>in vivo</i> immunomodulation noted in responsive group
Zhou et al. (2010)	4	42 (38–43)	Extensive, sclerodermal features	BM, third party	3–6/FBS	1–2 × 10 ⁷ MSC/kg; 4–8 intra-BM injections per patient	4/4 significant improvement; no infusional toxicity
Weng et al. (2010)	19	29 (18–39)	Extensive chronic	BM, third party	2–3/FBS	0.6 M/kg (range 0.2–1.4); 1–5 doses	74% ORR (4 CR, 10 PR), five patients able to stop immunosuppression, 2 year OS 78%; <i>in vivo</i> immunomodulation noted in responsive group



Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease[†]

Olle Ringdén^{1,±,*}, Tom Erkers¹, Silvia Nava Issue



The placenta protects the fetus from the mother's immune system. We have previously found that fetal membrane cells (FMCs) isolated from term placenta prevent alloreactivity in vitro. FMCs share many features with bone marrow-derived mesenchymal stromal cells (MSCs), which we previously introduced to treat severe acute graft-versus-host disease (GVHD). Here, we tested FMCs for treatment of steroid-refractory acute GVHD.

After two passages in culture, approximately 10^9 FMCs were obtained from one single placenta, although not all cells from passage 0 and passage 1 were used for expansion. The FMCs were positive for CD29, CD44, CD73, CD90, CD105, and CD49d but were negative for hematopoietic, endothelial, and epithelial markers. Microsatellite polymorphism analysis showed that FMCs were of maternal origin. All FMCs used showed normal karyotype.

Nine patients who had undergone hematopoietic stem cell transplantation and who had developed steroid-refractory grade III–IV acute GVHD were given $0.9\text{--}2.8 \times 10^6$ FMCs/kg at 15 infusions. Median age was 57 years. There was no toxicity from infusion of FMCs in eight patients. One patient had seizures after infusion. Two of eight evaluable patients had a complete response and four had a partial response, giving an overall response rate of 75%. Two patients showed no response at all. Three patients are alive from 6 to 21 months after HSCT. One patient is well and two have chronic GVHD.

Thus, FMCs may be successfully used for immune modulation and tissue repair.

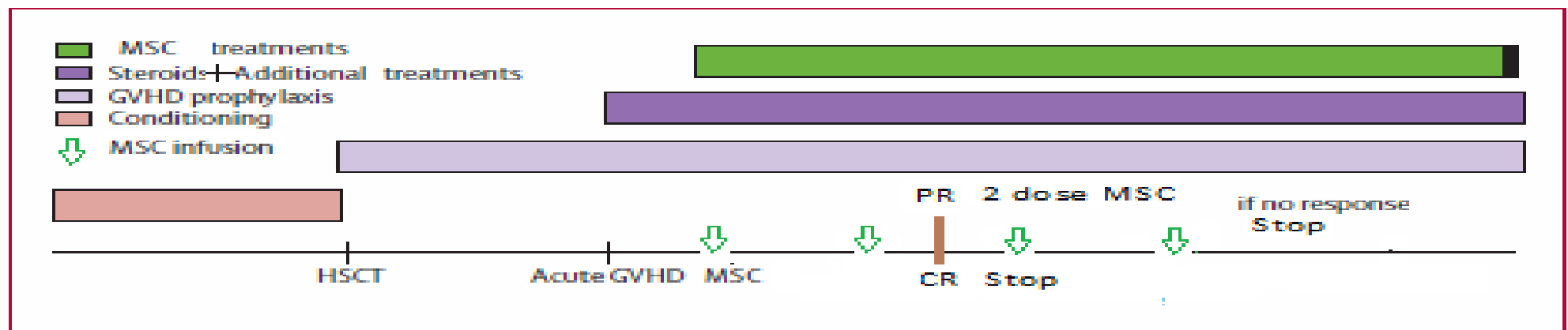
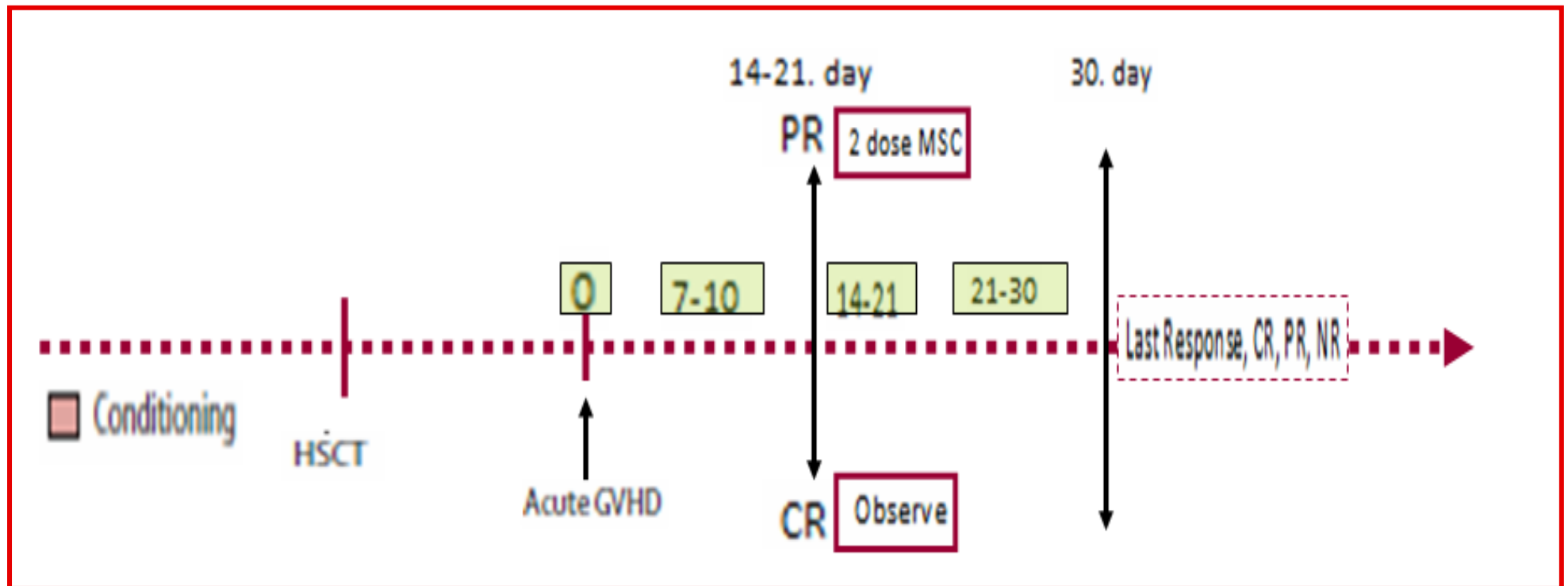
2013 Jan. [Epub ahead of print]

Important questions; which might impact the clinical efficacy of MSC as a cellular therapy for GvHD

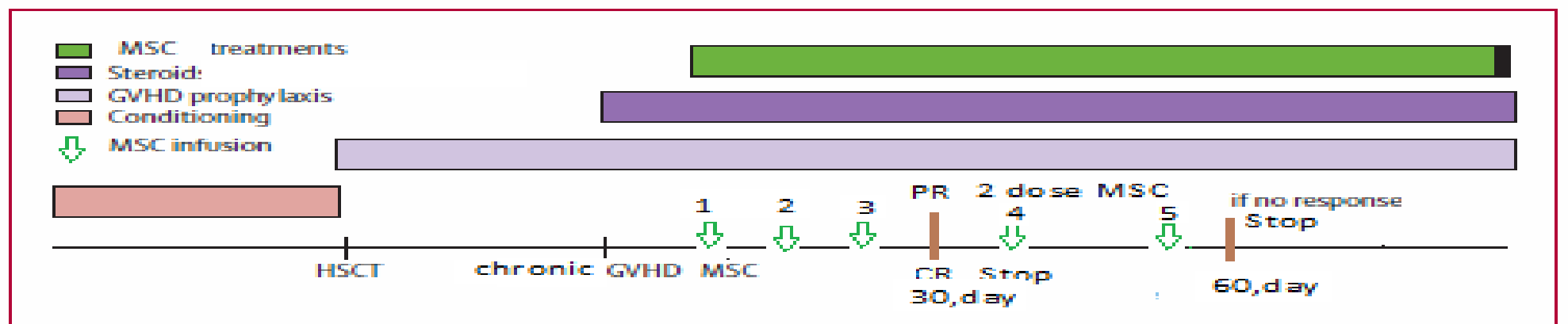
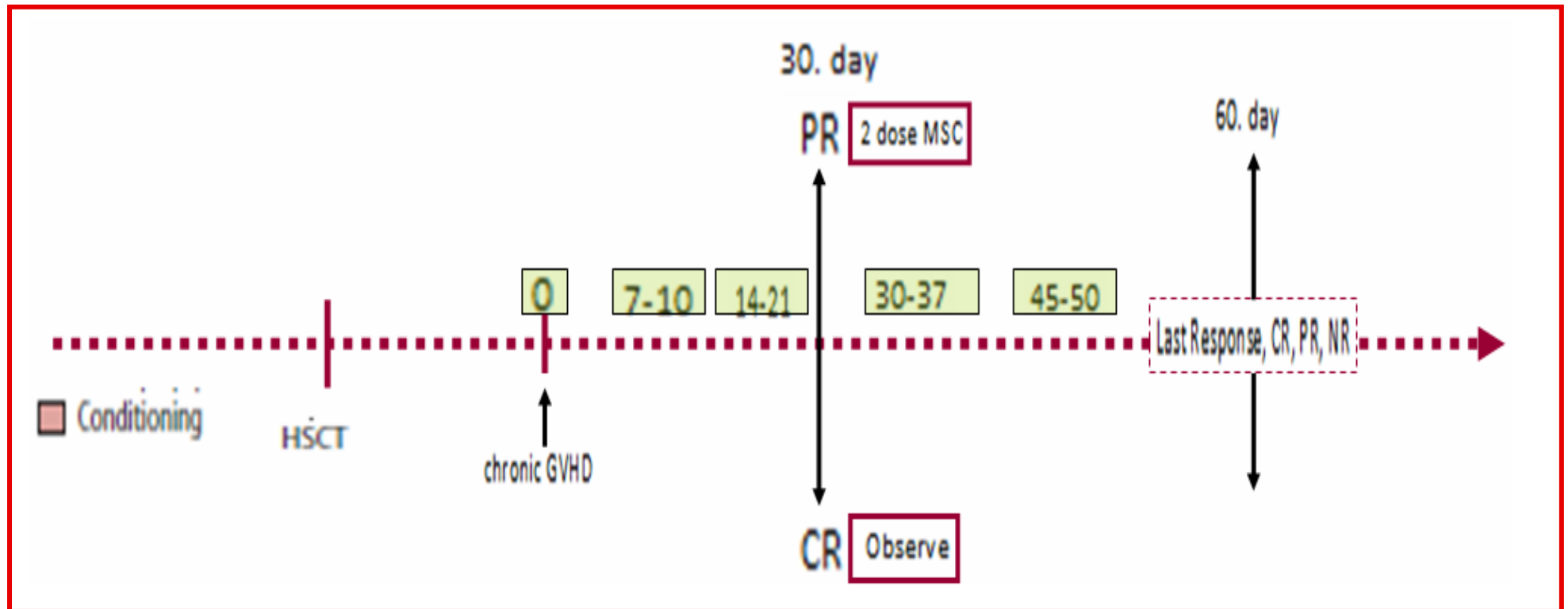
Such questions include:

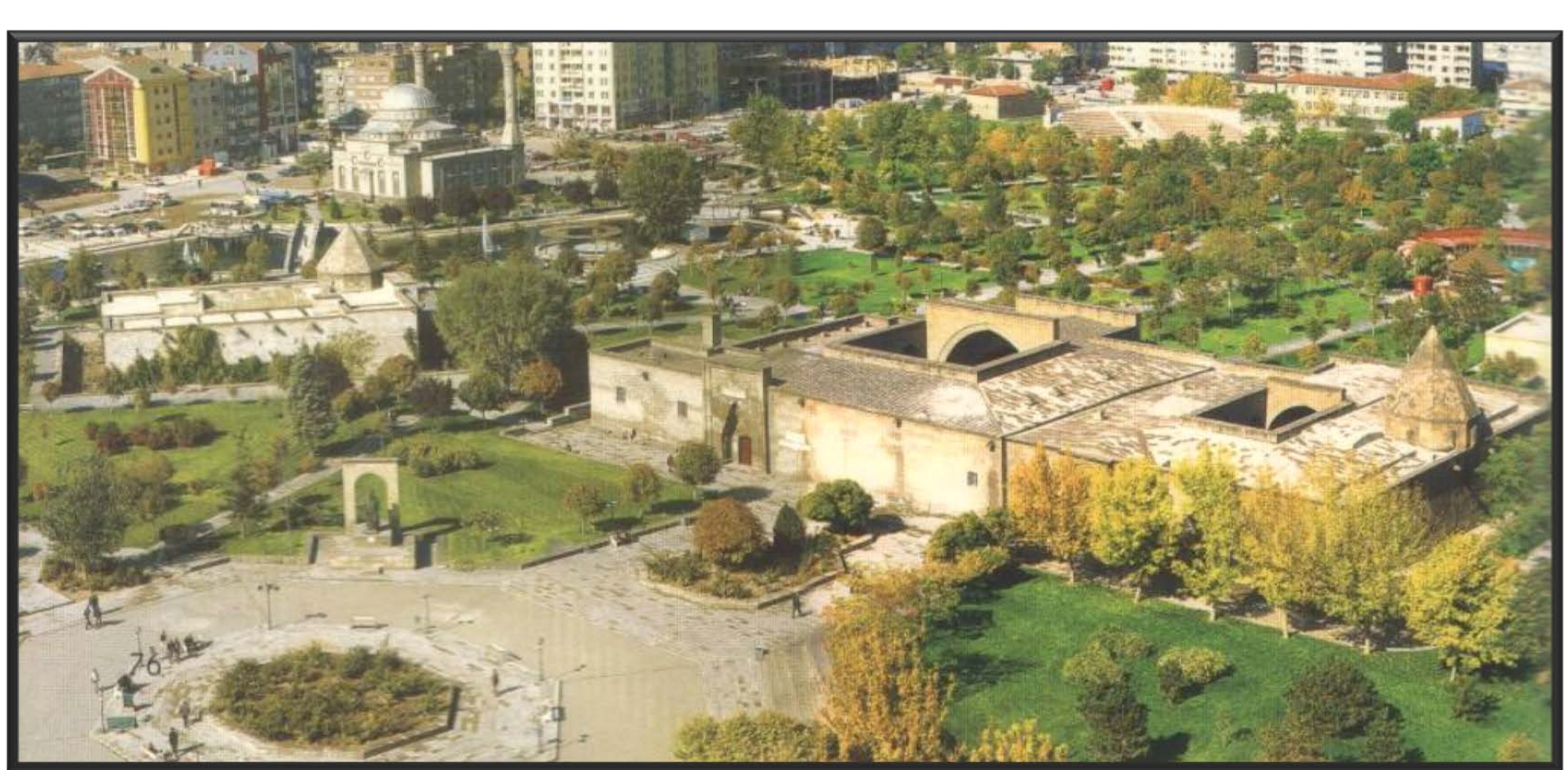
- (a) Defining the optimal dose of MSC,
 - (b) Determining the correct time for administration of MSC, and
 - (c) Studying the biodistribution of MSC.
-

MSC: Phase III Study in acute GVHD [MSC &ATG]



MSC: Phase III Study in chronic GVHD [MSC &ATG]





(Gevher Nesibe Madrasa: the first medical school)

In the Seljuks Periods: a large number of historical works of art such as mosques, madrasas

In That time ; thirty-two madrasas (universities) were maintained their education program

Kaniş-Karum (Kültepe) near the Kayseri: The center of Assyrian state

Kanish and Karum ruins, the first trade centers of the world A lot of trade agreement signed on stone tablets between Assyrian and Hittites & Egypt



Four thousand years before the first written trade agreement in Anatolia was made and written documents were found in the excavations of Kayseri Kültepe-Karum, 22 km to the east of KAYSERI.

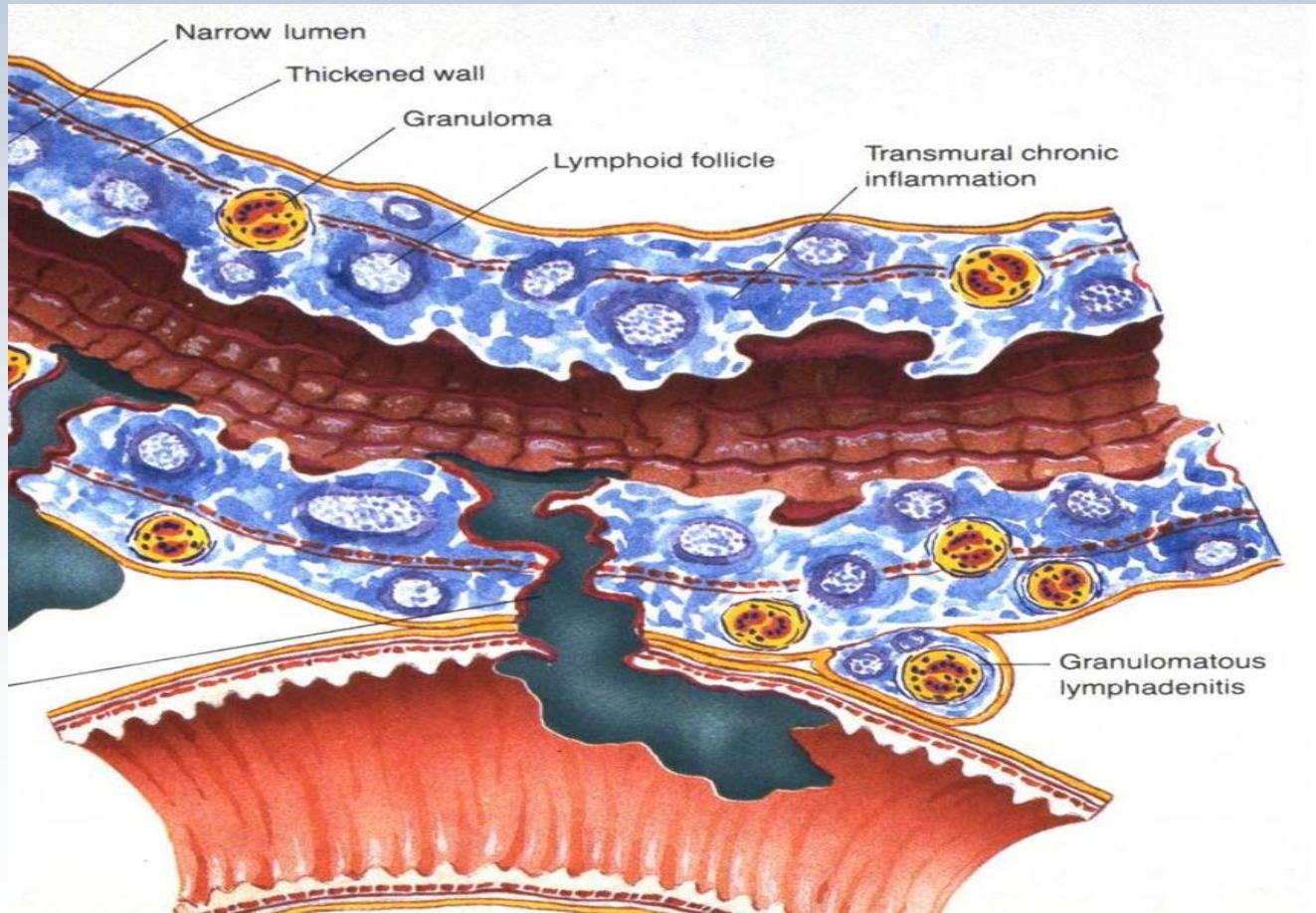




Mesenchymal Stem Cell treatment for autoimmune diseases: a critical review

Mesenchymal stem cells (MSCs) are now known to display not only stem cell multipotency, but also robust antiinflammatory and regenerative properties. After widespread *in-vitro* and *in-vivo* preclinical testing, autologous and allogeneic MSCs have been applied in a range of immune mediated conditions, including graft versus host disease, Crohn's disease, multiple sclerosis, refractory systemic lupus erythematosus and systemic sclerosis. Current data suggests that MSCs may not only replace diseased tissues, but also exert several trophic, regenerative and antiinflammatory effects. While the clinical outcome in case reports and phase I-II trials seems occasionally striking, these limited results point to the need to perform controlled multicenter trials. Future advances from stem cell science can be expected to pinpoint significant MSC subpopulations and/or stem cell markers for improved regenerative or immunoregulatory properties.

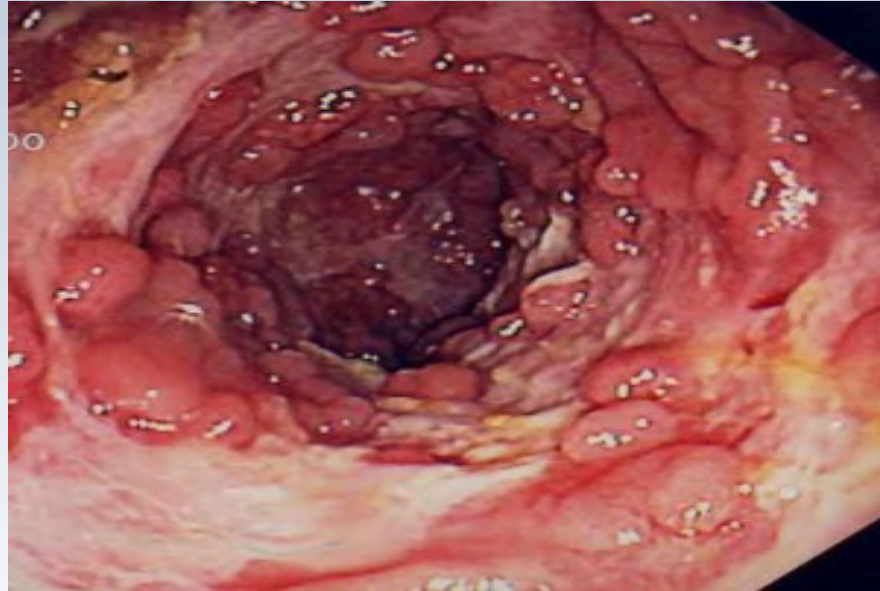
Crohn Disease; Crohn's disease is an inflammatory bowel disease



Crohn causes inflammation of the lining of your digestive tract, which can lead to abdominal pain, severe diarrhea and even malnutrition.

Crohn Disease;

involve the entire gastrointestinal tract with persistent transmural inflammation and fistulization.



- The first report of a phase I clinical trial of cell therapy using autologous adipose-derived MSCs was published in 2005. **Local injection led to healing of fistulas (6/8) with no adverse effects** (Garcia-Olmo et al.)
- Onken et al. reported a clinical response (**≥ 100 point reduction in the Crohn Disease Activity Index**) in **3/9 (33.3%)** patients, (2006)

Systemic Lupus Erythematosus (SLE)

SLE is a chronic autoimmune disease that can affect almost any organ system; thus, its presentation and course are highly variable, ranging from indolent to fulminant

Perhaps the most successful results of human MSC therapy emerge from clinical trials aimed at severe treatment refractory SLE (Liang et al., 2010).



Butterfly-shaped rash

- *Tögel et al. reported that remarkable improvement renal conditions and the other SLE symptoms of SLE (2009)*
- *A second trial from this group in China, (Sun et al., 2010), reporting the use of umbilical cord-derived MSCs in severe lupus patients (n=16). Follow-up was only 8.25 months, but significant improvement was verified for SLEDAI score, serum albumin, 24h urinary protein, serum creatinine, serum complement and anti-dsDNA antibodies.*

ORIGINAL ARTICLE

Mesenchymal SCT ameliorates refractory cytopenia in patients with systemic lupus erythematosus

X Li¹, D Wang¹, J Liang, *Bone Marrow Transplantation* advance online publication, 15 October 2012; doi:10.1038/bmt.2012.184

- 35 SLE patients with refractory cytopenia
- 20 patients had leukopenia, 24 with anemia or thrombocytopenia.
- The average follow-up period after MSCT was 21 months
- Significant improvements in blood cell count were found after MSCT
- Most patients, in parallel with the decline of disease activity.

ORIGINAL ARTICLE

Mesenchymal SCT ameliorates refractory cytopenia in patients with systemic lupus erythematosus

X Li¹, D Wang¹, J Liang, *Bone Marrow Transplantation* advance online publication, 15 October 2012; doi:10.1038/bmt.2012.184

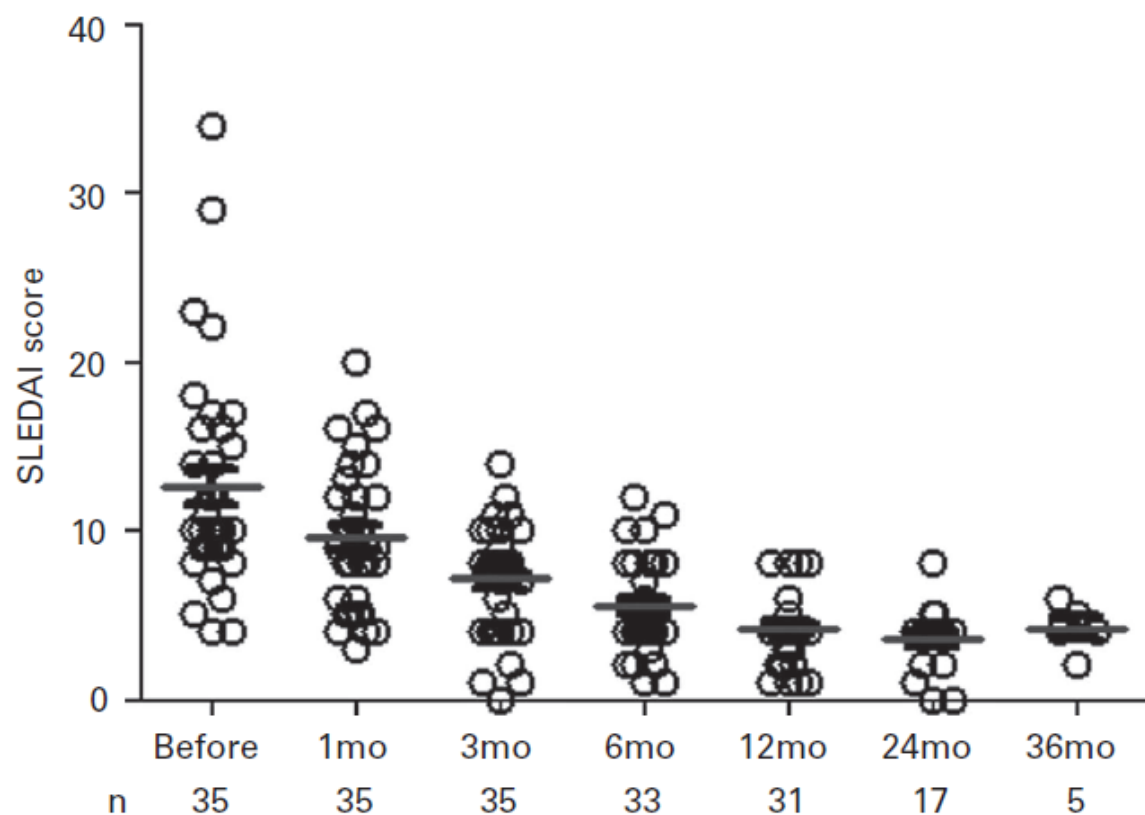
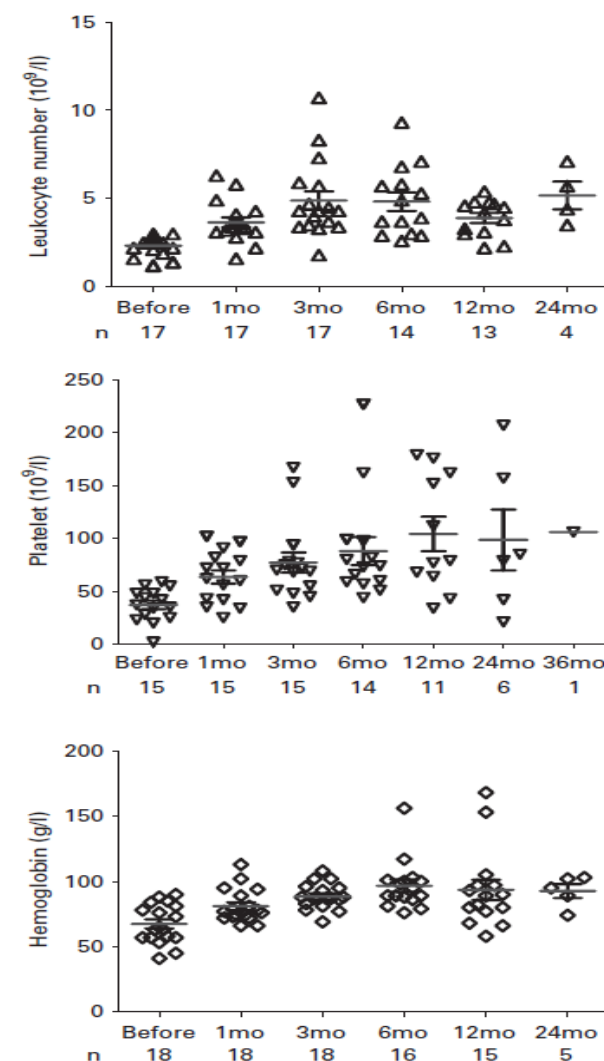


Figure 2. SLE Disease Activity Index (SLEDAI) score for each patient pre- and post- MSCT. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.



Systemic Sclerosis (SS)

SSc is a systemic autoimmune connective tissue disease.

Characteristics of disease include vasomotor disturbances; fibrosis; subsequent atrophy of the skin, subcutaneous tissue, muscles, and internal organs (eg, alimentary tract, lungs, heart, kidney, CNS);

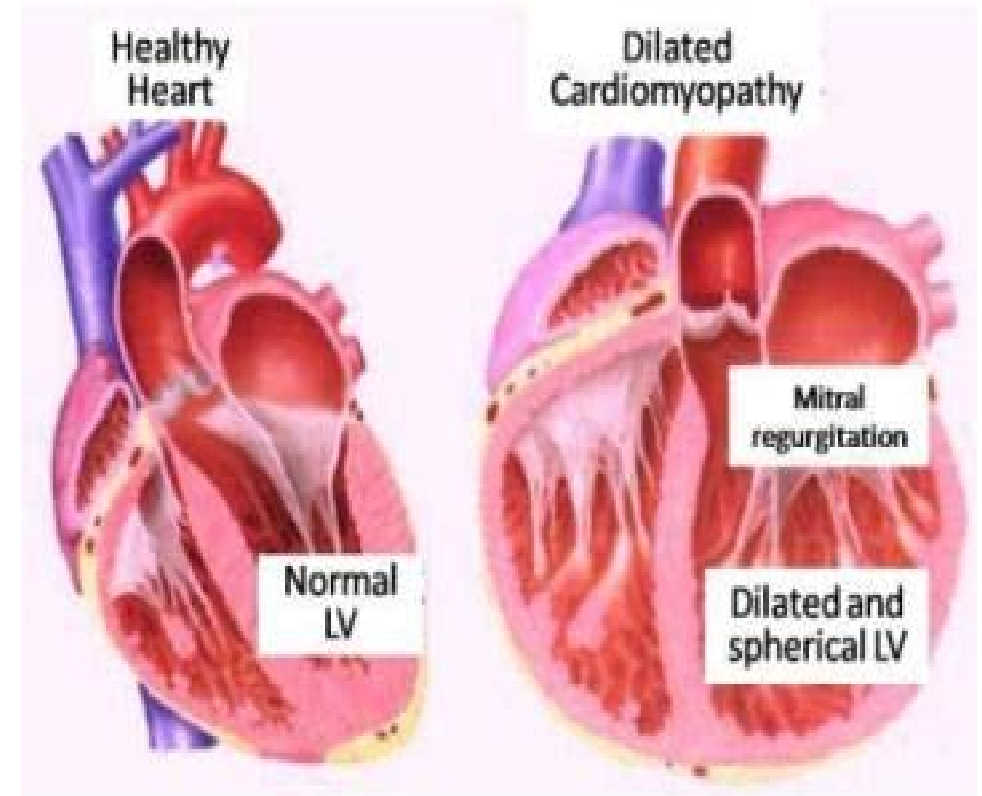
- In a most interesting investigation by Akiyama
- Transplantation of allogeneic MSCs in 5 patients with SS
- Triggered the induction of T cell apoptosis,
- Triggered the lymphopenia and Treg induction
- Leading to skin ulcer healing in one case

Significant improvement in the Skin Score, Health Assessment Questionnaire and autoantibody titer in the whole group. (Guiducci et al., 2007) (Akiyama et. al., 2012).



ISCHAEMIC CARDIOMYOPATHY

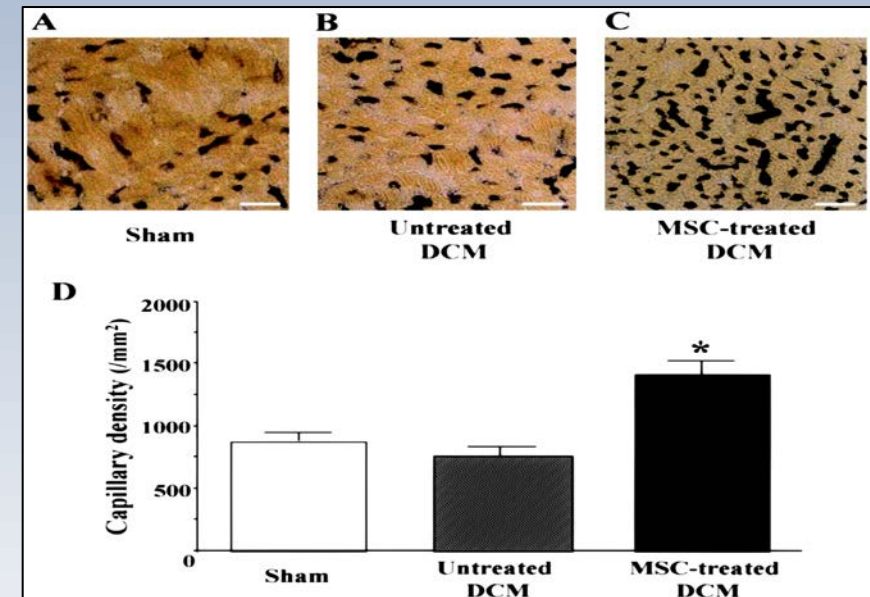
- MSCs have also been used in with promising results .
- However, the benefits of MSC treatment may be due to paracrine effects of MSCs instead of their capacity to differentiate into cardiomyocytes after injection.



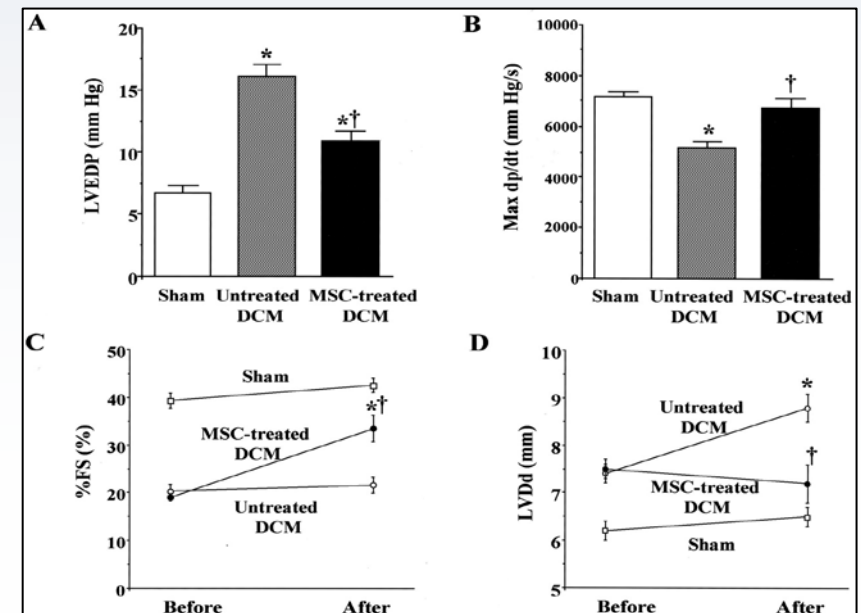
MSCs for cardiovascular repair

Nagaya N et al found that MSC transplantation

- In a rat model of dilated cardiomyopathy
- Significantly increased capillary density
- Decreased left ventricular end-diastolic pressure
- Increased left ventricular maximum



Representative samples of ALP staining of myocardium.



Effects of MSC transplantation on hemodynamic parameters.

Clinical trials using MSCs to improve cardiac function have also demonstrated encouraging results

- In a pilot study, sixty-nine patients who underwent primary percutaneous coronary intervention within 12 hours after onset of acute myocardial infarction
- They were randomized to receive intracoronary injection of autologous bone marrow mesenchymal stem cell or standard saline.
- Several imaging techniques demonstrated that MSCs significantly improved left ventricular function [22].

Chen SL, et al: Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 2004, 94:92–95.

TABLE 2 Comparison of Left Ventricular Hemodynamics in the Two Groups of Patients

Variables	BMSC Group	Control Group	p Value
Patients (n)	34	35	0.20
Functional defect (%)			
Just before BMSC implantation	32 ± 11	33 ± 10	0.20
At 3-mo follow-up	13 ± 5	28 ± 10	0.001
Infarcted area movement velocity (cm/s)			
Just before BMSC implantation	2.17 ± 1.3	2.19 ± 1.5	0.20
At 3-mo follow-up	4.2 ± 2.5	2.7 ± 1.7	0.01
Left ventricular ejection fraction (%)			
Just before BMSC implantation	49 ± 9	48 ± 10	0.20
At 3-mo follow-up	67 ± 11	53 ± 18	0.01
At 6-mo follow-up	67 ± 3	54 ± 5	0.01

TABLE 3 Cardiac Functional Indexes at Three-month Follow-up in the Two Groups of Patients

Variables	Control Group	BMSC Group	p Value
Patients (n)	35	34	0.20
LV ESV (ml)	162 ± 27	136 ± 31	0.001
LV ESV (ml)	88 ± 19	63 ± 20	0.01
Circumferential shortening (mm/s)	21.7 ± 5.9	24.8 ± 4.2	0.10
P _{sys} /ESV (mm Hg/ml)	2.84 ± 1.30	1.72 ± 1.23	0.01
Perfusion defect by PET (cm ²)	185 ± 87	134 ± 66	0.001

ESV = end-systolic volume; LV = left ventricular; PET = positron emission tomography; P_{sys} = left ventricular end-systolic pressure.

TABLE 4 Cardiac Functional Index by NOGA System in the BMSC Group

	Pretransplantation (n = 15)	At 3-mo Follow-up (n = 15)	p Value
Line local shortening (%)	7.32 ± 1.86	11.29 ± 1.64	0.01
Unipolar voltage (mV)	7.61 ± 1.09	10.38 ± 1.12	0.01
Perfusion defect (%)	36 ± 6	20 ± 5	0.01
Stroke volume index (%)	40 ± 11	58 ± 10	0.01
LV end-diastolic volume (ml)	169 ± 21	131 ± 19	0.01
LV end-systolic volume (ml)	76 ± 18	58 ± 13	0.01

LV = left ventricular.

Phase 1 Trial of Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Decompensated Liver Cirrhosis

In a phase I trial, four patients with decompensated liver cirrhosis were included. They received autologous MSC infusion through a peripheral vein. There were no side-effects in the patients during followup. The quality of life of all four patients improved by the end of follow-up

Mohamadnejad M. Arch Iran Med 2007, 10:459-466.

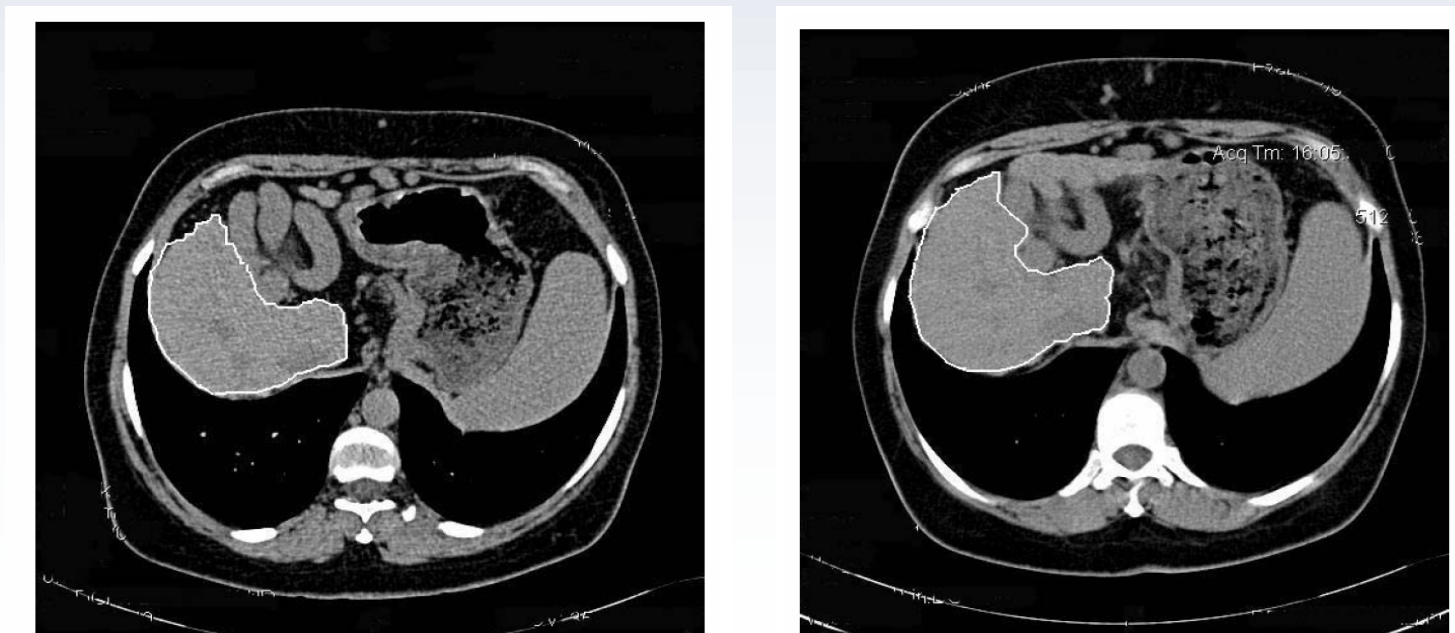


Figure 2. A) Transverse slices of CT scans obtained before, and B) Six months after MSCs infusion in patient I. White outline in CT scans indicates the right liver lobe.

Phase 1 Trial of Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Decompensated Liver Cirrhosis

Table 2. Clinical and laboratory parameters of the patients at baseline and at the end of follow-up.

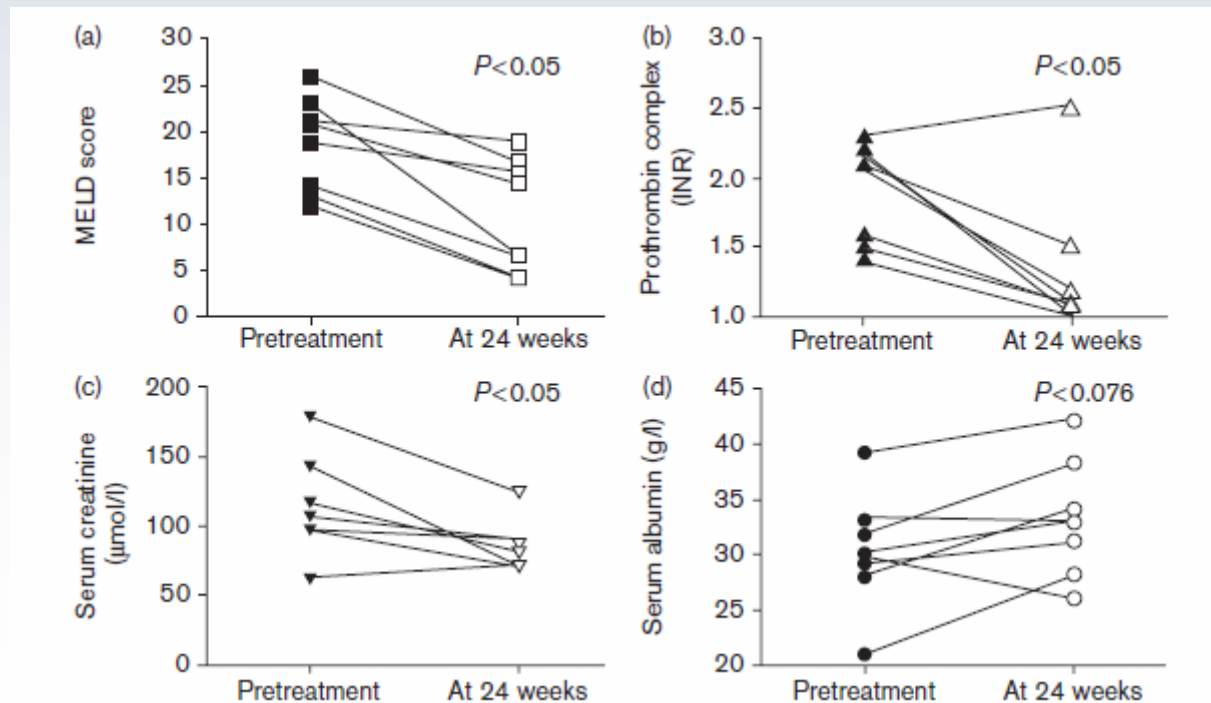
Parameter	Patient 1			Patient 2			Patient 3			Patient 4		
	B	M 6	M 12	B	M 6	M 12	B	M 6	M 12	B	M 6	M 12
Edema*	2+	0	0	2+	1+	1+	2+	0	1+	2+	0	0
Ascites	None	None	None	None		None	Mild	None	None	None	None	None
Serum albumin (g/dL)	2.9	3.8	3.5	2.8	2.9	3	4.1	4.3	3.9	3.4	3.2	3.2
PT (seconds)	18.5	13	15.2	20.1	18.8	18	15.7	15.1	15.5	19.1	13.8	15.5
INR	2.2	1.2	1.6	2.01	2	1.7	1.4	1.3	1.4	1.9	1.1	1.4
Cr (mg/dL)	0.7	0.88	0.80	1.1	0.74	1	0.55	0.85	0.66	0.73	0.92	0.70
Total bilirubin (mg/dL)	1.3	2	0.9	2.7	5.32	3.2	4.43	3.25	3.33	2.18	1.9	2.6
Direct bilirubin (mg/dL)	0.4	0.5	0.5	1	0.91	1.2	1.7	1.25	1.24	0.44	0.49	0.39
AST (IU/mL)	66	149	77	67	59	49	63	74	49	39	43	33
ALT (IU/mL)	53	127	52	24	34	23	81	71	57	25	30	17
AFP (µg/L)	2.6	2	5.5	4.1	2.5	5.5	2.5	2	2	1.5	2.3	0.66
MELD score	16	11	12	19	20	20	16	14	15	17	10	14
Liver volume (cm ³)	495	814		555	843		1115	872		795	940	—

PT=prothrombin time; INR=international normalized ratio; Cr=serum creatinine; AST=aspartate aminotransferase; ALT=alanine aminotransferase; AFP=alpha fetoprotein; MELD=model for end-stage liver disease, B=baseline; M=month.

*Peripheral edema was graded as follows: 0=no edema; trace=indentation caused by pressure over the dorsum of the foot; 1+ =indentation at shin; 2+ =indentation at knee; 3+ =indentation above knee; 4+ =generalized edema (indentation over hip and low back).

Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial

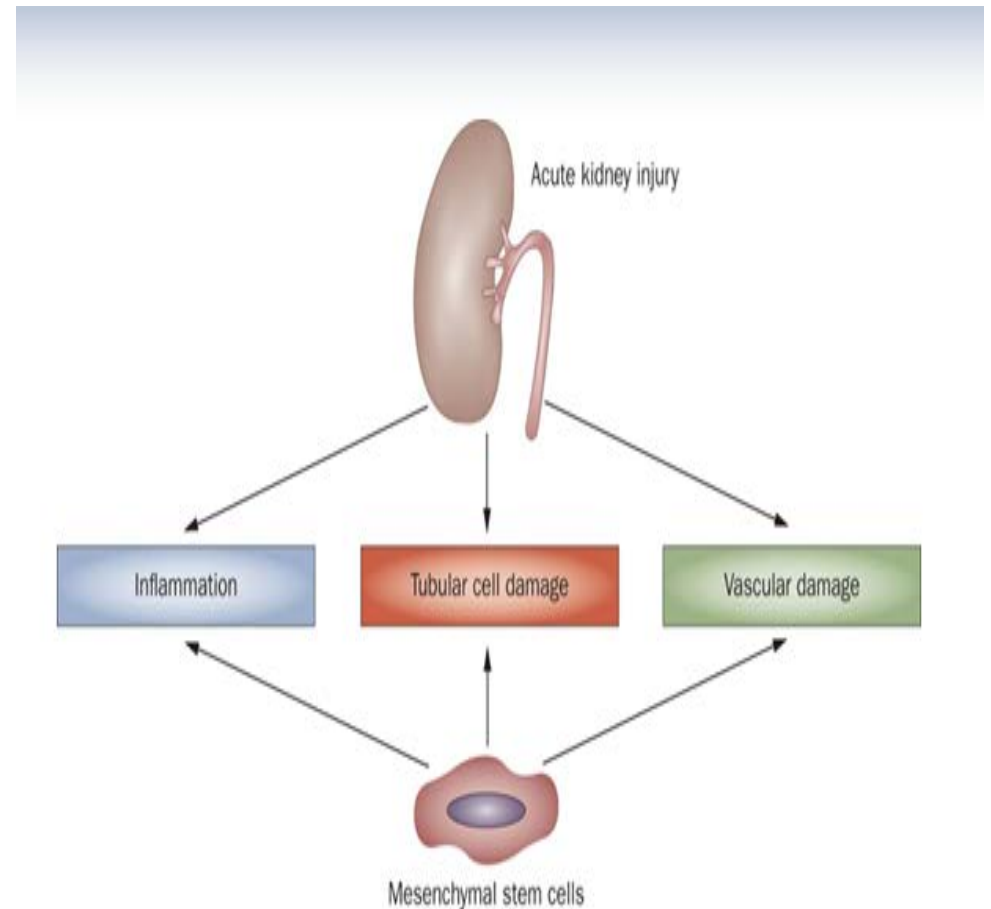
- In another phase I-II clinical trial, 8 patients (four hepatitis B, one hepatitis C, one alcoholic, and two cryptogenic) with end-stage liver disease were included.
- After autologous MSCs injection, all patients tolerated well and their liver function improved, suggesting the feasibility, safety, and efficacy of using MSCs as a treatment for end-stage liver disease



Diagrams of liver function indices before and after injection of mesenchymal stem cells. (a) Model for End-Stage Liver Disease (MELD) score. (b) prothrombin complex. (c) Serum creatinine. (d) Serum albumin. Filled symbols denote pretreatment values; open symbols denote values 24 weeks posttreatment. INR, international normalized ratio.

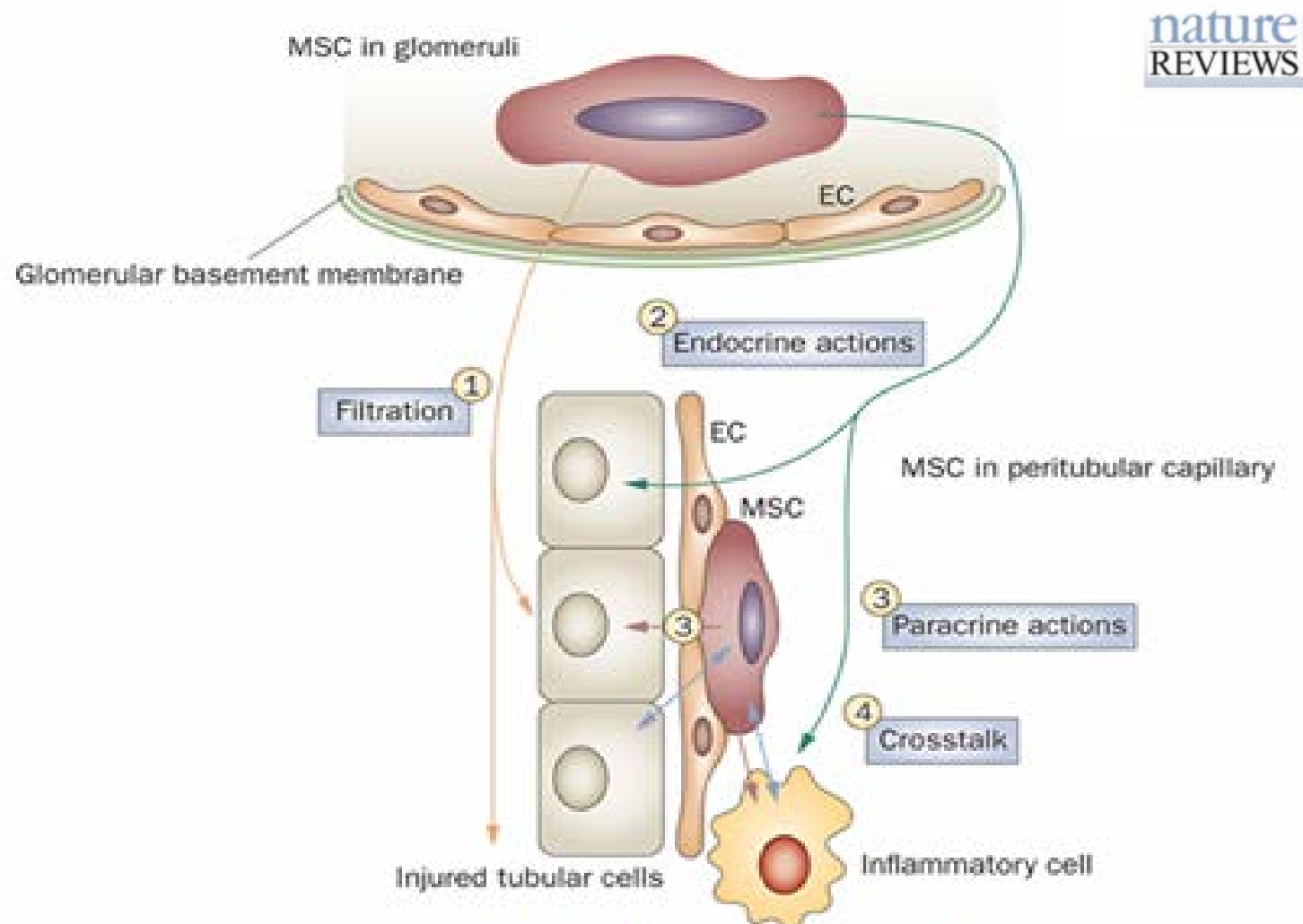
Mesenchymal stem cells target all pathophysiological components of acute kidney injury

- Major axes of the pathophysiology of AKI include inflammation, vascular and tubular damage. Inflammation is triggered by ischemia–reperfusion injury, inflammatory cytokines, and immune cell attachment and migration.
- Vascular damage is caused by ischemia–reperfusion. Endothelial injury and further microcirculatory impairment aggravates tubular cell damage and increases inflammation.
- Tubular cell injury is caused by hypoxia and by the generation of reactive oxygen species during reperfusion



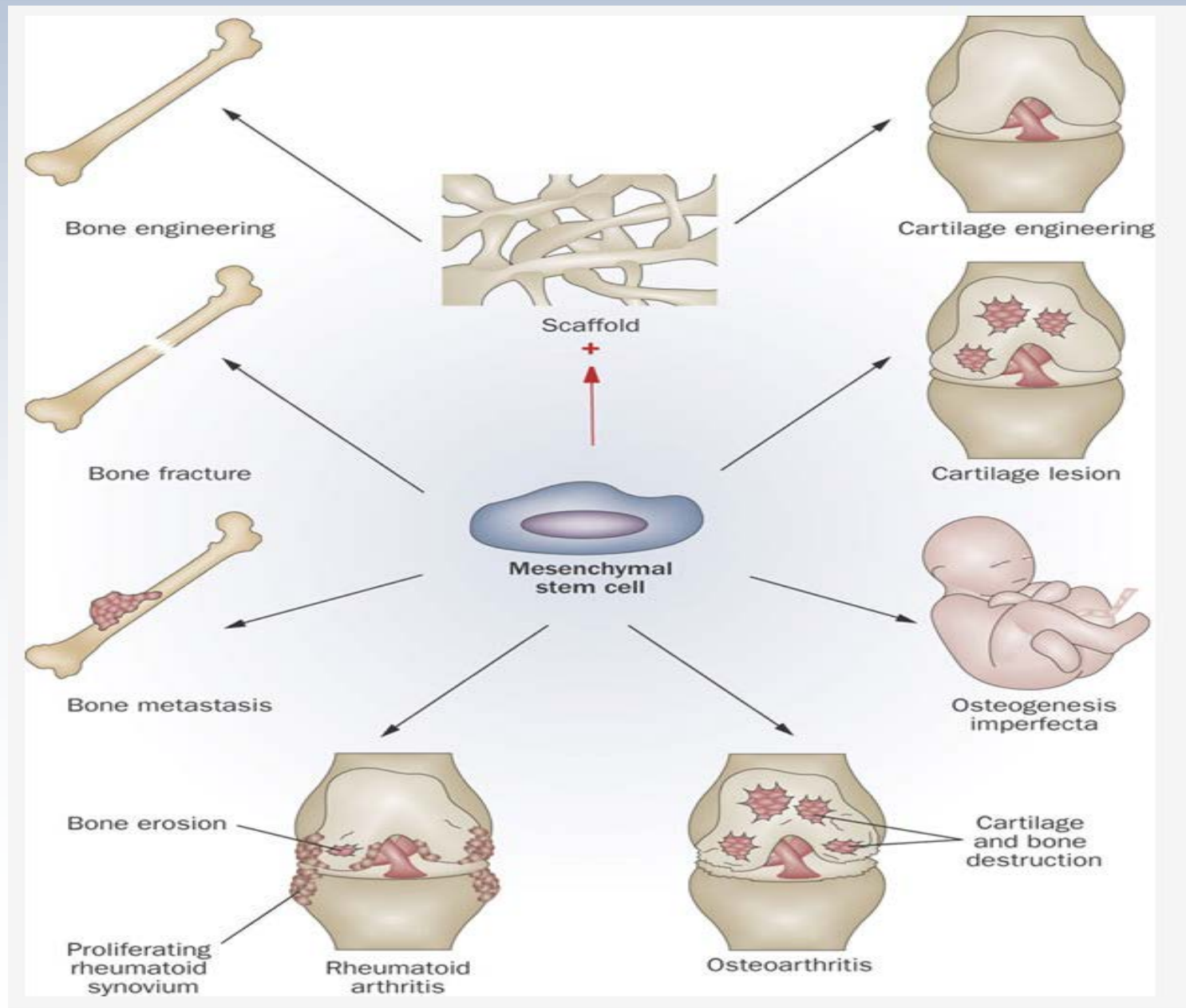
Nat. Rev. Nephrol. doi:10.1038/nrneph.2009.229

Intrarenal actions of MSCs in acute kidney injury

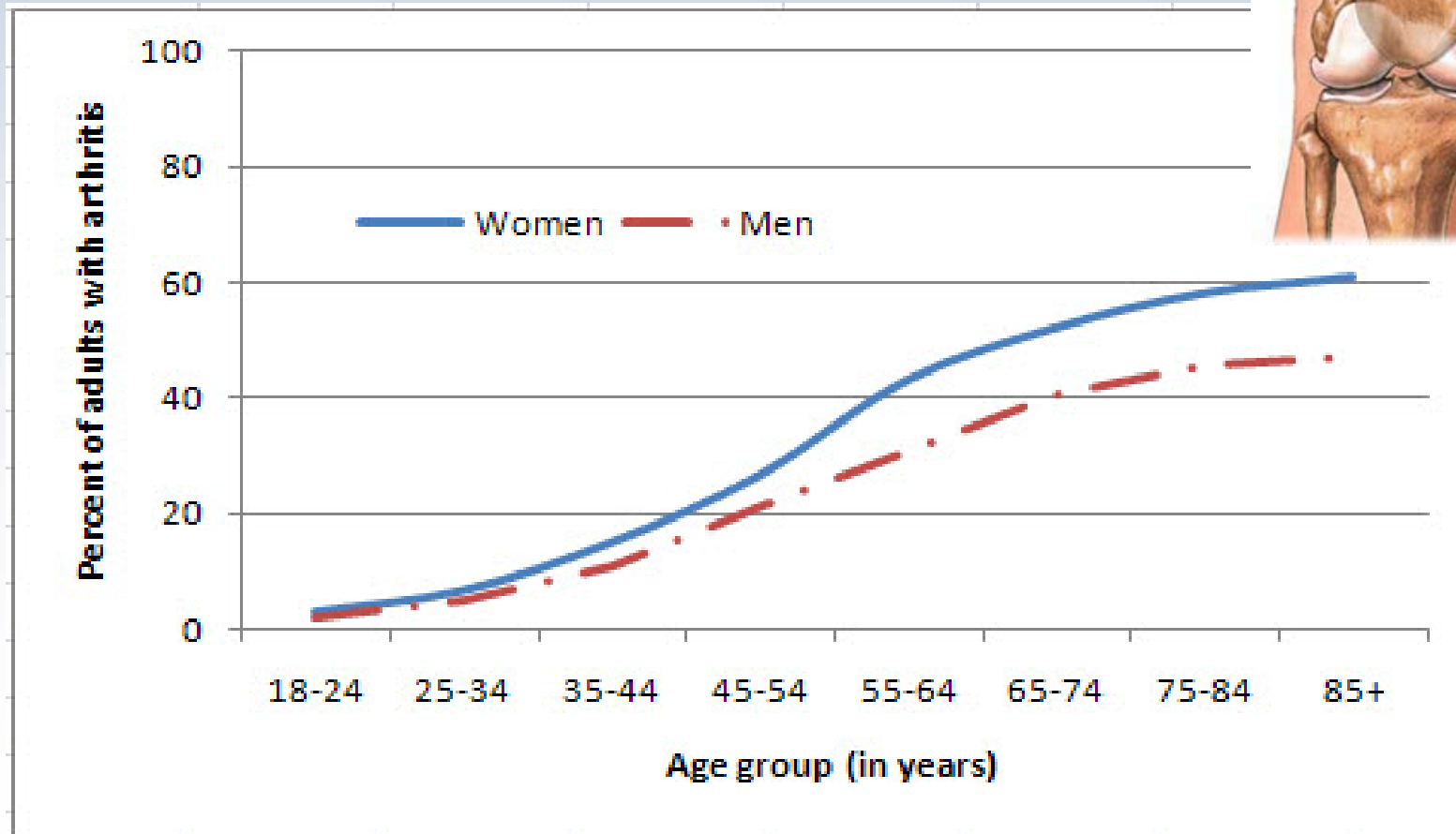


Tögel, F. E. & Westenfelder, C. (2010)

Orthopaedic Disorders and Mesenchymal Stem Cell Therapy



Arthritis in the United States



Current Treatment Options

- **Drugs**

- NSAID (Ibuprofen, Naproxen)
- Painkillers (Narcotic and Non-Narcotic)
- Corticosteroids

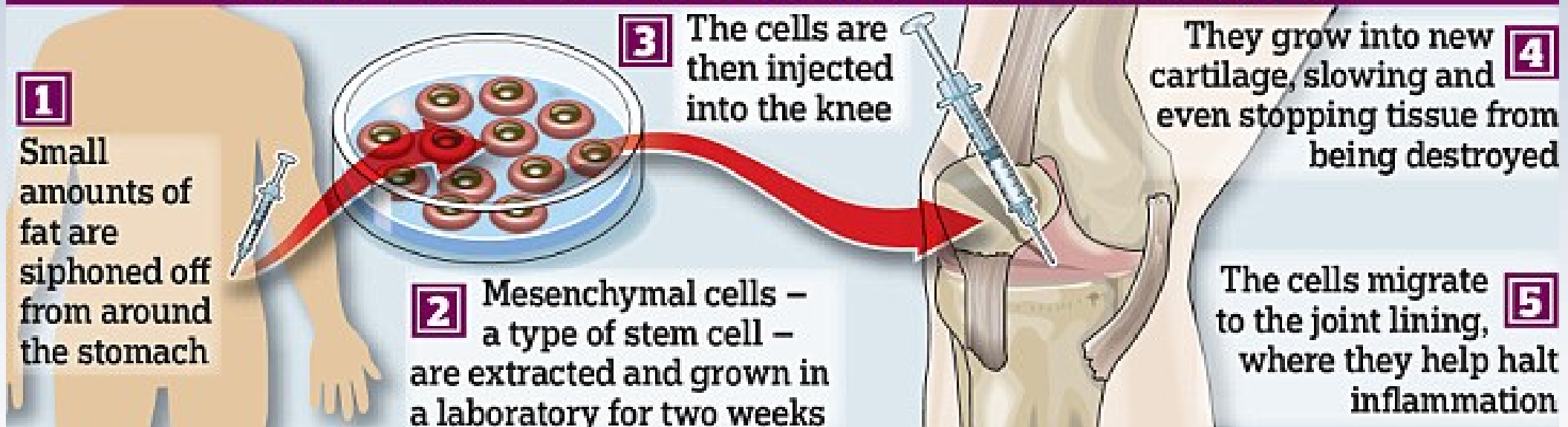
- **Joint Surgery**

- Knee replacement, hip replacement

Perpetual or Extremely Invasive

Future Treatment Options

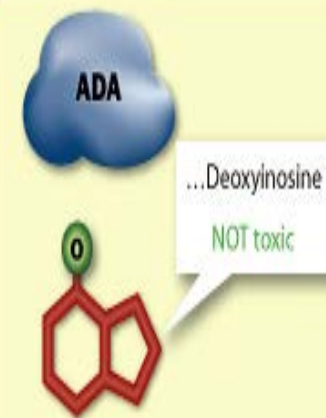
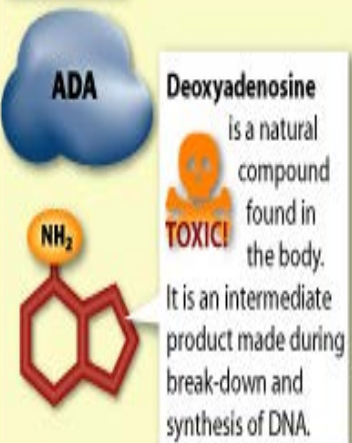
HOW THE TREATMENT WORKS



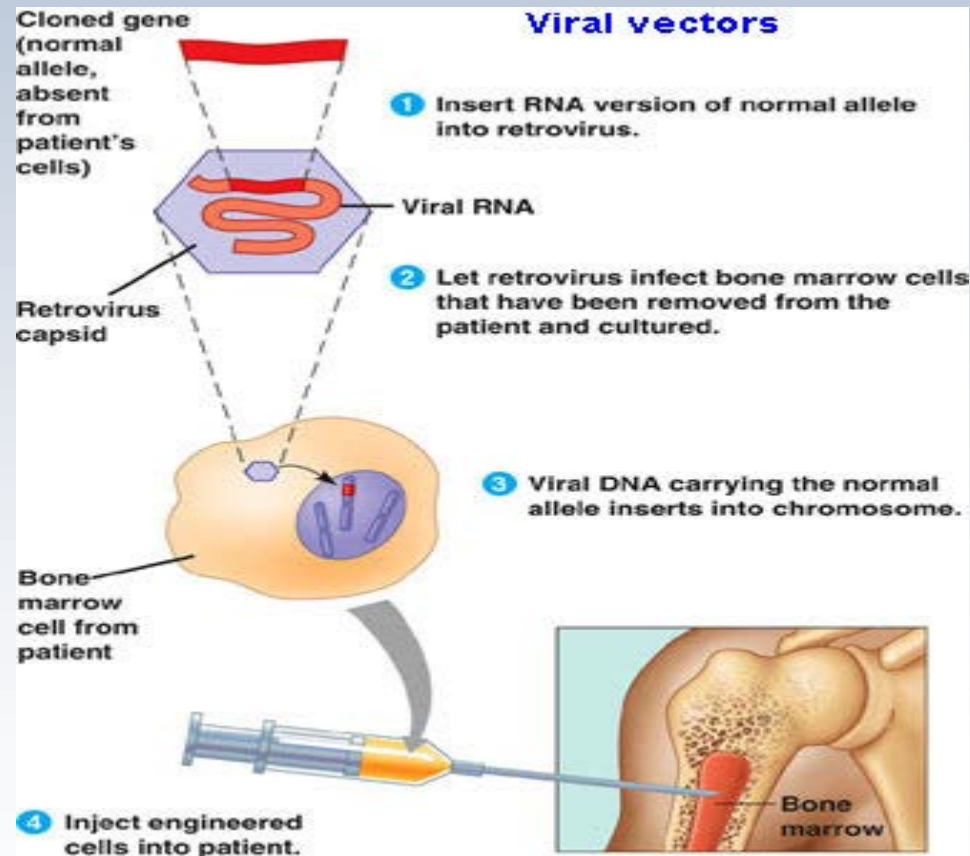
- Minimally Invasive without the daily dose of drugs
- **We are almost there!**

ADA Converts Deoxyadenosine to a Non-toxic Substance

Normal

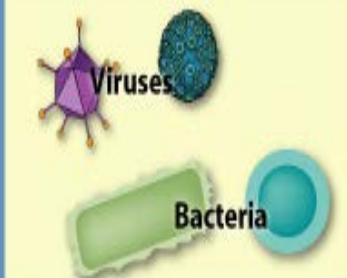
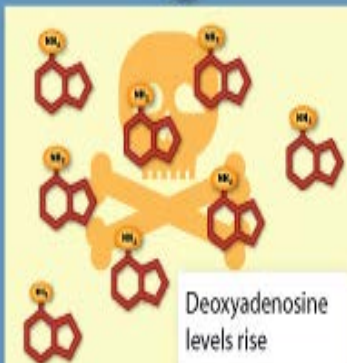
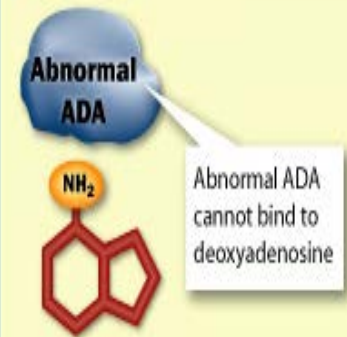


ADENOSINE DEAMINASE DEFICIENCY



Gerson et al 1997 implanted subcut MSC graft transduced with a functional adenosine deaminase gene

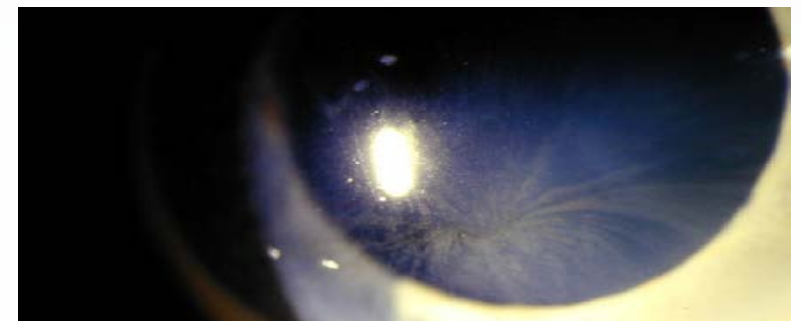
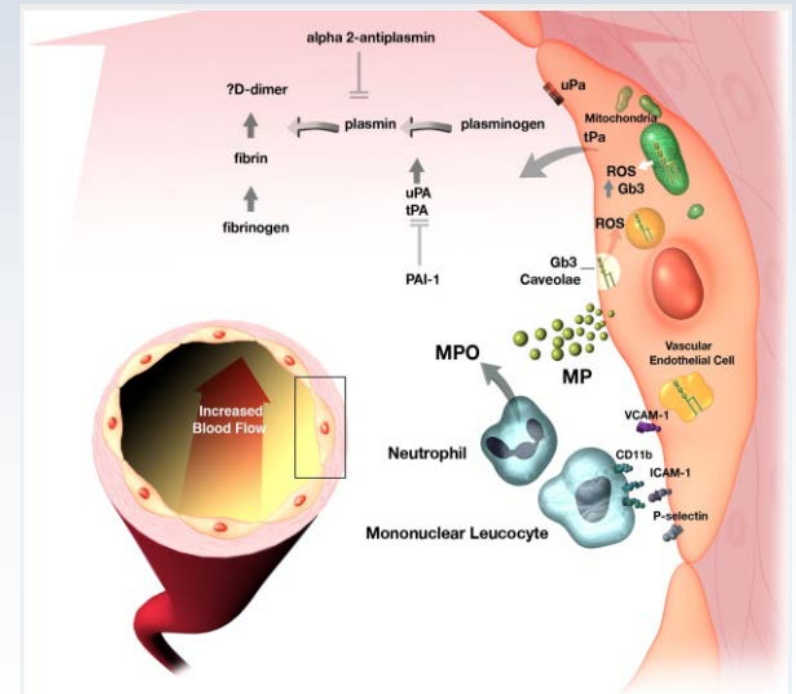
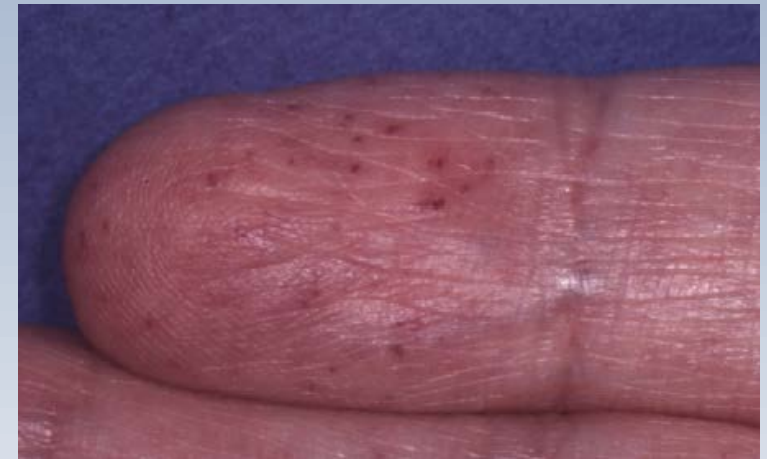
ADA Deficiency



- High levels of deoxyadenosine kill B and T cells of the immune system
- The body is open to infection by bacteria and viruses

FABRY'S DISEASE

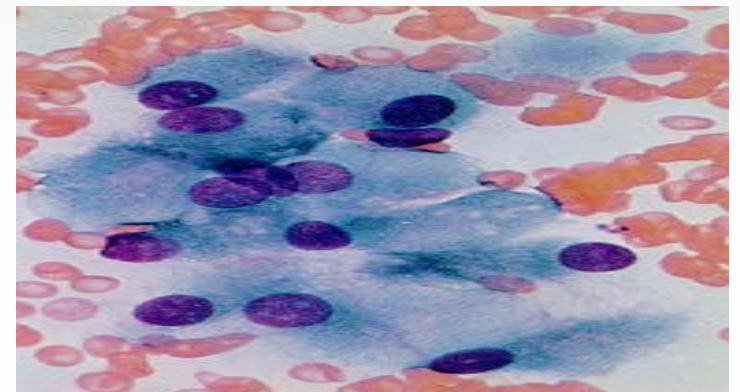
- X-linked genetic disorder - deficiency of lysosomal enzyme alpha-galactosidase
- Using patient's own MSC
- Transduced with a functional galactosidase gene
- Return MSC to the patient
- Correction of deficiency (Osiris, 2000)



OSTEOGENESIS IMPERFECTA

- Horwitz et al 1999 reported 3 children transplanted with allogeneic MSC from HLA-compatible siblings
- New lamellar bone formation, improved osteogenesis with fewer fractures
- Engrafted MSC were shown to differentiate into osteoblasts

E. M. Horwitz, D. J. Prockop, A. Lorraine et al., "Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta," *Nature Medicine*, vol. 5, no. 3, pp. 309–313, 1999



Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi

Ali Ünal , Leylagül Kaynar , Naci Emiroğulları , , Mustafa Çetin

- Küçük ve orta çaplı arter ve venleri tutan nonaterosklerotik, segmental, inflamatuvar, obliteratif bir damar hastalığıdır.



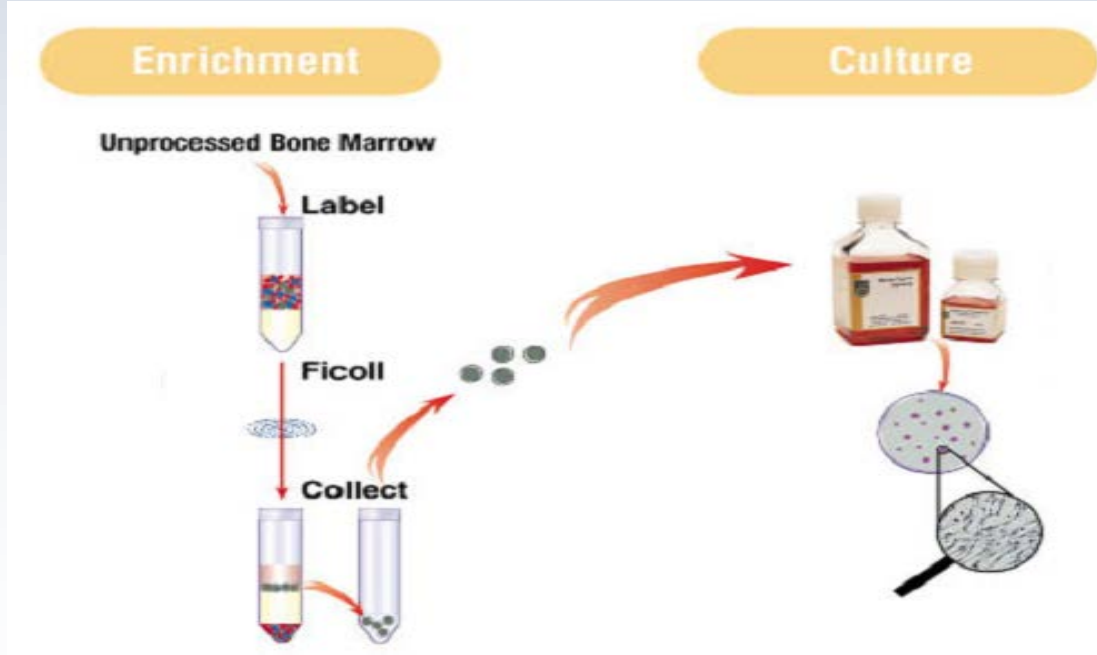
- Çoğu vakada standart tedavi ile semptomlar ilerlemekte ve amputasyonla sonuçlanmaktadır.

Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi

- 34 yaşında erkek hasta.
- Altı yıl önce Burger Hastalığı tanısı konulmuş ve vasküler yetersizlik nedeniyle gelişen kangren sonucu 4 yıl önce sağ ayak 3,4 ve 5. parmakları ile sol ayak 2. parmağı ampüte edilmişti.
- Tıbbi tedavi olarak aspirin, heparin ve dipridamol tedavisi almış
- İki yıl önce her iki bacakta, Femoral ve popliteal arterlere By Pass operasyonu uygulanmış.
- Bu tedavilere rağmen, klinik ve laboratuvar olarak yeterli sonuç elde edilememiş.



Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi



- Her iki bacak ve ayaklarında ülserasyonları olan hastaya, mezenkimal kök hücre tedavisi uygulandı.
- Mezenkimal kök hücreler, kemik iliği kök hücrelerinden elde edildi.

Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi

- ATİ teknoloji laboratuvarında, , hasta serumu ve sitokinler içeren kültür ortamında, kemik iliği kök hücrelerinden, mezenkimal kök hücreler üretildi.
- Mezenkimal kök hücreler, anestezi altında sol bacak gastrektnemius kası içerisine enjekte edildi.
- Üretilen mezenkimal kök hücre sayısı ancak bir bacak için yeterli olduğundan, sol bacağa tedavi uygulandı,
- sağ bacağa ise tedavi uygulanamadı.

Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi

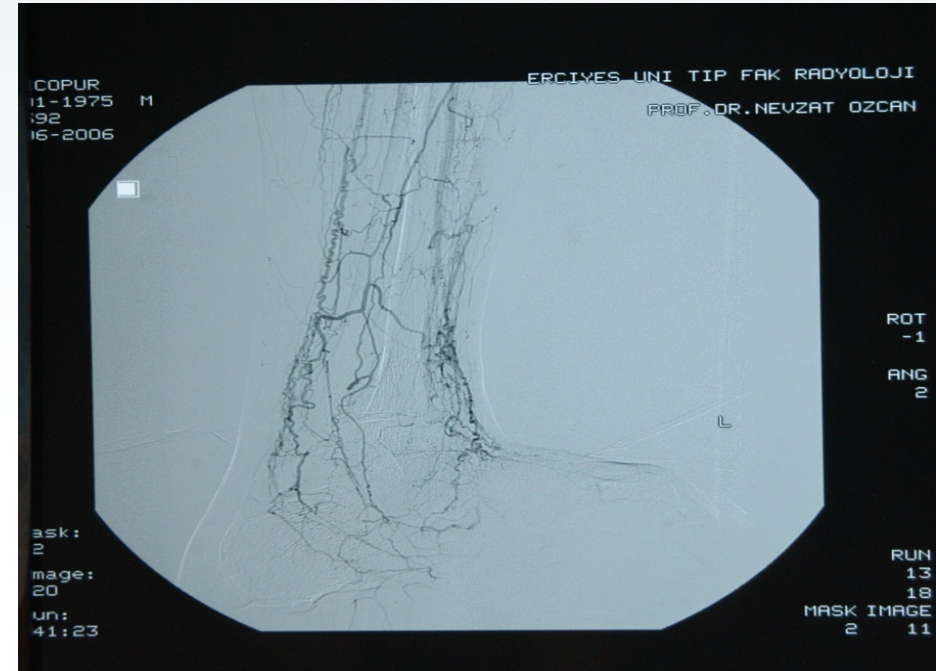
Mezenkimal kök hücre tedavisinden sonra; Sol bacakdaki ülserasyonların kaybolduğu gözlemlendi. Tedavi yapılmıyan sağ bacak ve ayakta, ülsere lezyonlar devam ediyordu.

Klinik olarak karşılaştırıldığında; Mezenkimal kök hücre tedavisi uygulanan sol bacakta, yürüme ile gelişen ağrı ve krampların azaldığı tesbit edildi. Sağ bacakta ağrı, kramp ve yürüme güclüğü devam ediyordu.



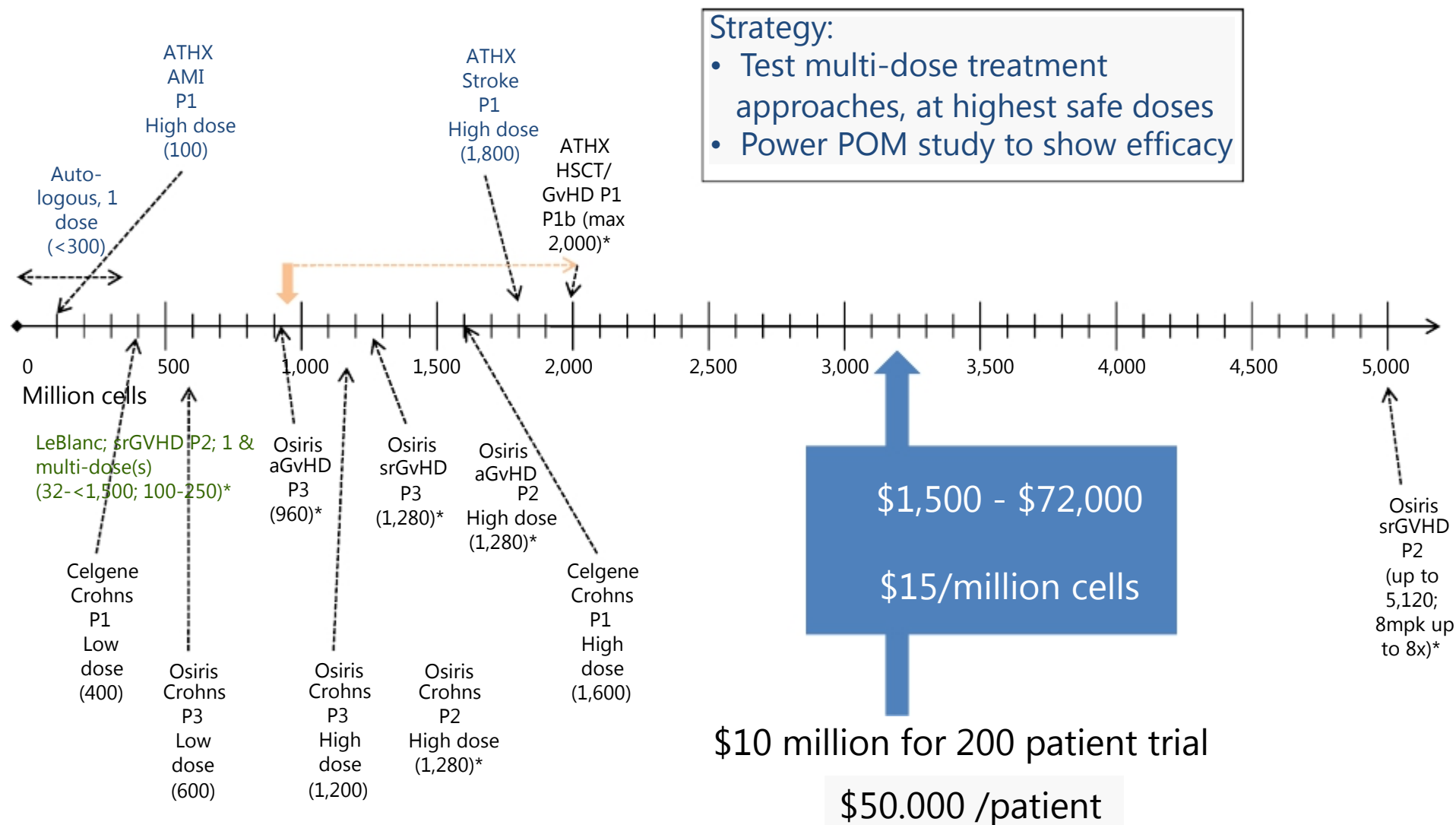
Burger Hastalığı (Tromboangiitis obliterans)' da Mezenkimal Kök Hücre Deneyimi

Kök hücre tedavisi sonrası çekilen anjiyografi ile sol bacakta yeni damar oluşumları tesbit edildi.



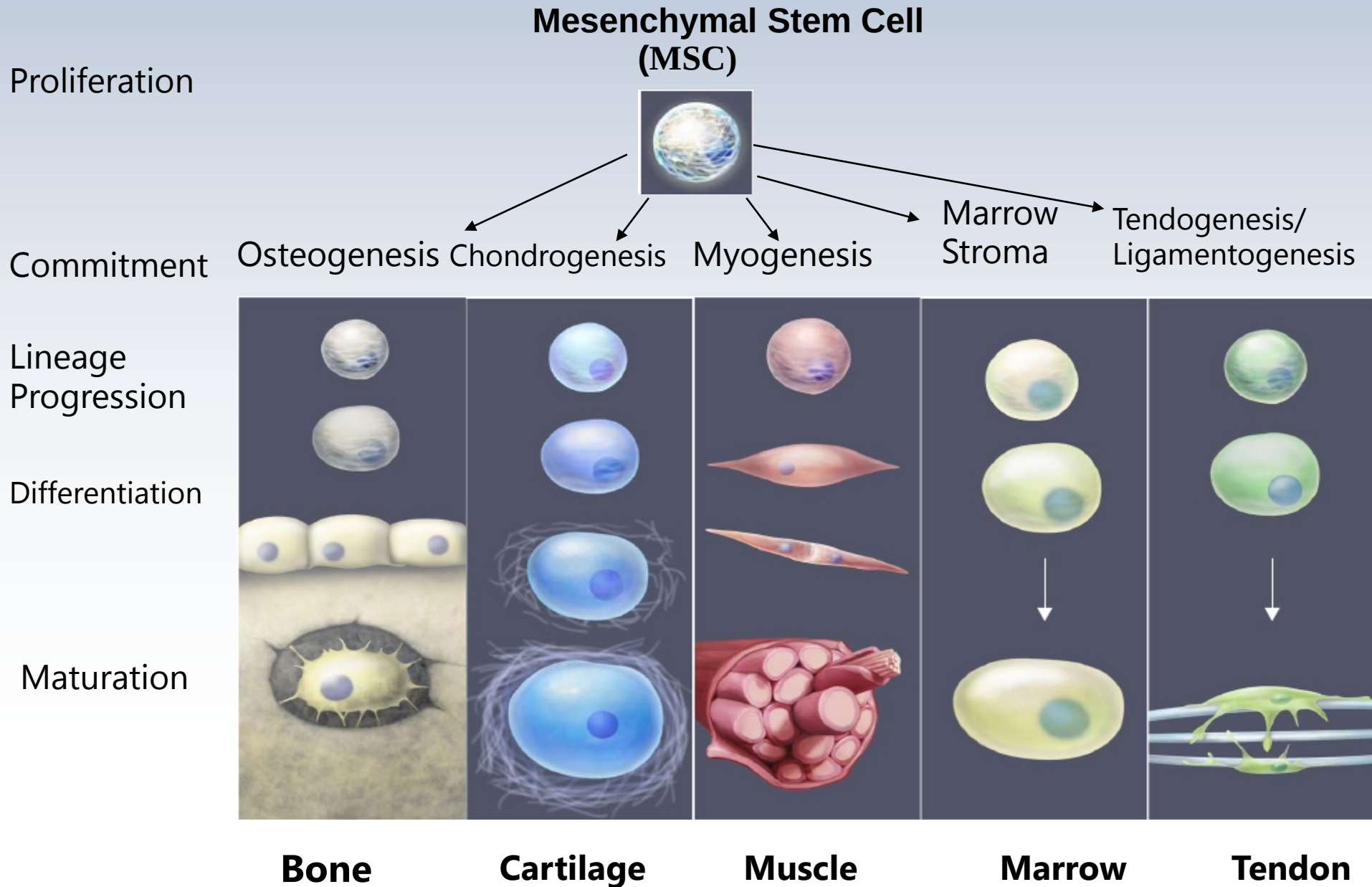
Cumulative Dose per Patient – Selected Clinical Studies

Wide Range for Treatment COGS: Reimbursable?



* Assumes 100 kg patient

The Mesengenic Process



What Are Our Biggest Development Challenges?

- Clinical product characterization and potency
 - Lack of standards and clearing house for comparability
 - How does a physician select between products?
 - How does a physician determine comparability between sites?
- Regulatory agencies are asking for potency measurements including institutional patient designated products
- Improving dose and treatment regimen decisions
 - Need for new technologies and approaches to determine PK/PD profile
- Bringing development experience and investment capital in from pharma and healthcare
 - Investments guarded to date

Summary

- MSC therapies extending rapidly and globally in new indications
 - General consensus on Regulatory standards through ISCT, EBMT
- Safety profile good, clinical proof of concept still lacking
- Key Phase III approval trials launched(ing)
 - GVHD treatment (EBMT, Osiris)
 - Crohn's (Osiris, Cellerix)
 - PVD (Aastrom)
 - CHF (Mesoblast)
- Critical investment in late stage clinical development still lacking from pharma and healthcare industry
- Fundamentals underlying dose selection and treatment regimens need attention
 - Need for stronger science in PK/PD (**Pharmacokinetic/pharmacodynamic**) profiling of therapies

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- Kayseri, Erciyes University Hospital (BMT), CIC 627, A (140 142) 77/63)
- 3th at the list of European Bone Marrow Numbers.



Thank you for your attention