College of Medicine & Health Sciences
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Mesenchymal Stem Cells to Repair Vascular Damage after Chemotherapy: Past, Present and Future

Cell Therapy 2014

Las Vegas, NV, USA

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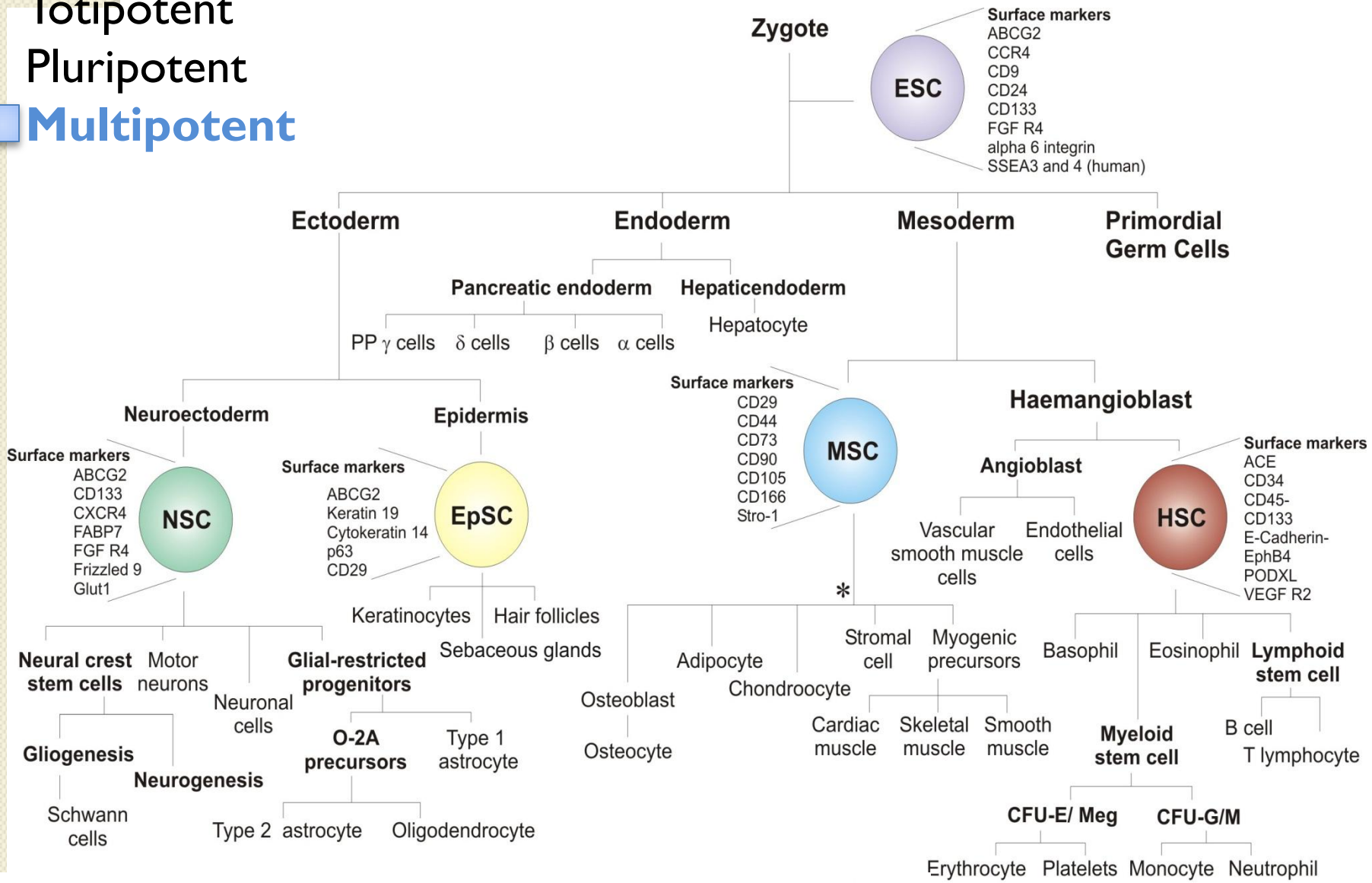
What are MSCs?

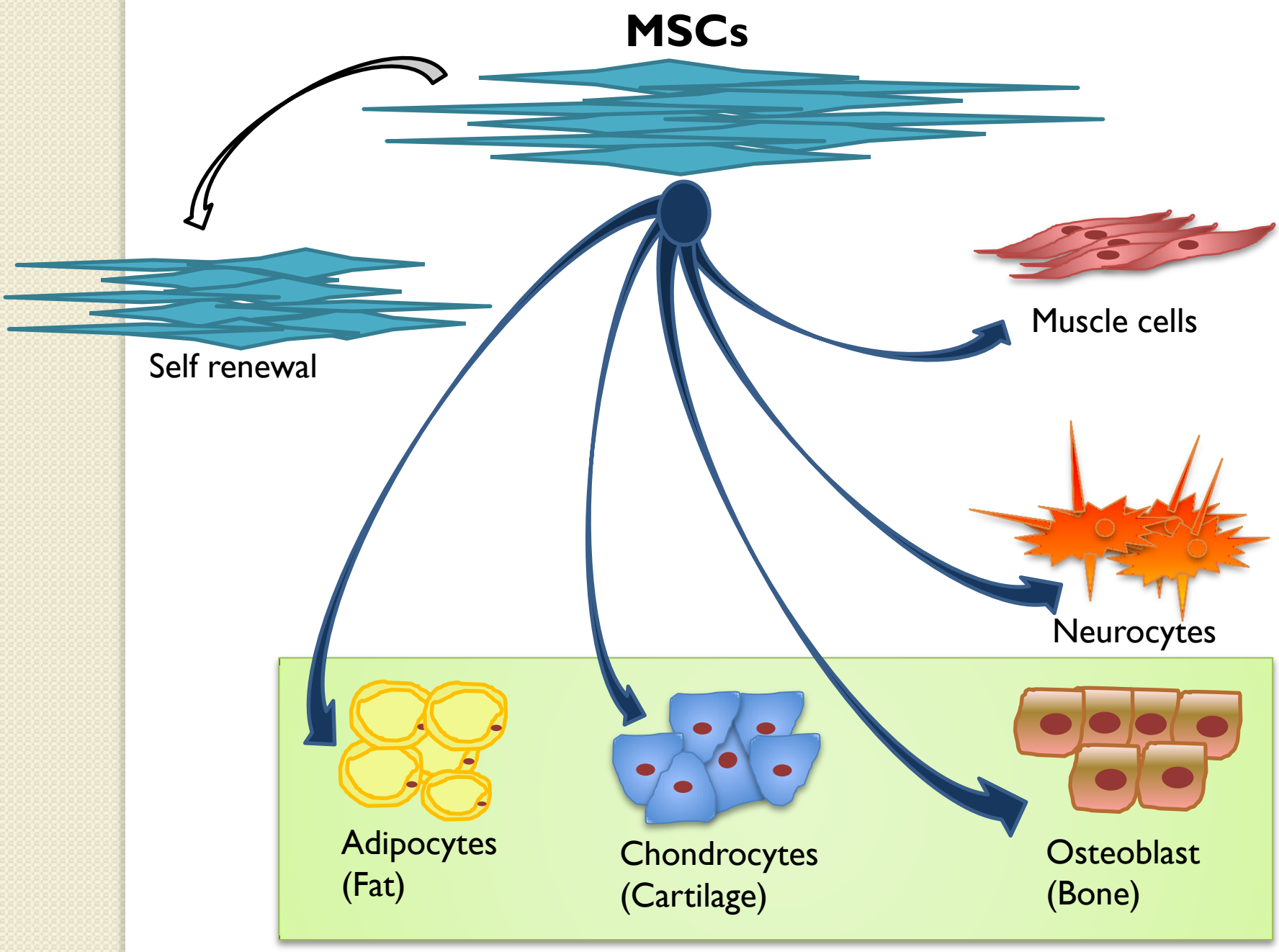
- Stem cell capacity
- Stromal property
- Non-hematopoietic cells
- Multipotent cells
- 0.001 - 0.01% of nucleated cells in BM
- Adhere to culture plate
- Express variable range of cell markers

- Totipotent
- Pluripotent
- **Multipotent**



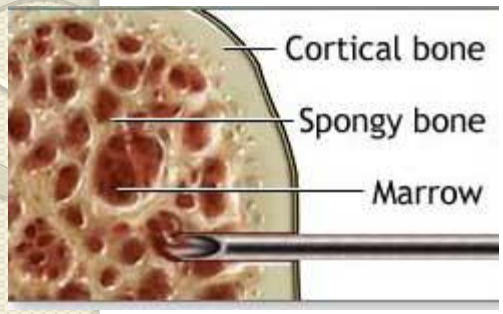
Development of stem cells within the body. This is not a comprehensive diagram, for clarity only selected stem cells are shown . ESC = embryonic stem cell, NSC = neuronal stem cell, EpSC = epidermal stem cell, MSC = mesenchymal stem cell, HSC = haematopoeitic stem cell. *Differentiation of MSCs along neuronal lineages has also been demonstrated, see text for information. Modified from R&D Systems website (http://www.rndsystems.com). Copyright BTR©

http://www.york.ac.uk/res/bonefromblood/background/osteogenesis.html


MSCs
Self renewal
Muscle cells
Neurocytes
Adipocytes
(Fat)
Chondrocytes
(Cartilage)
Osteoblast
(Bone)

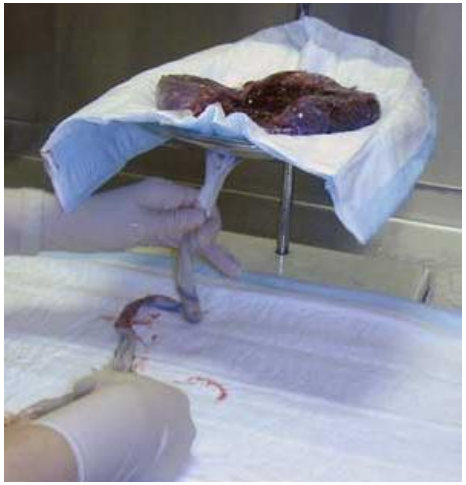
MSC Sources

Bone marrow

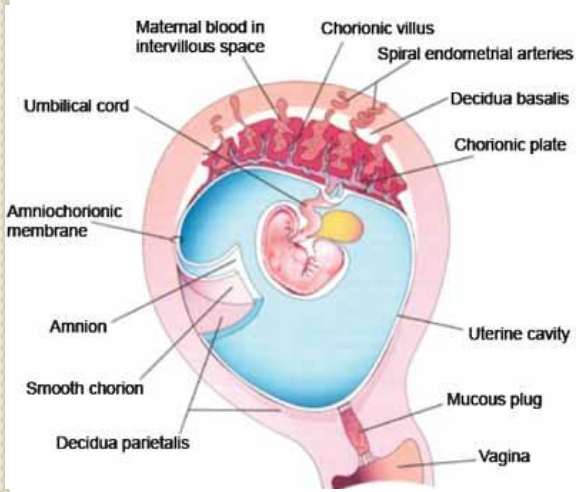


Peripheral blood

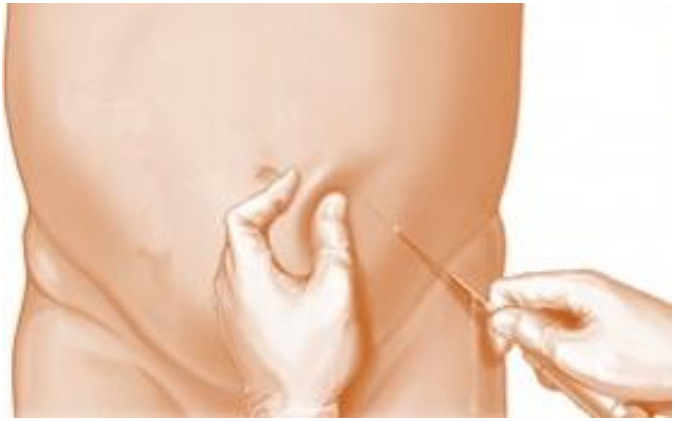
Cord blood



Placenta and fetal membrane



Adipose tissue



Hass *et al* 2011; Orbay *et al* 2012

Common MSC Markers

Negative Markers	**Roles**
CD34	Primitive hematopoietic cells and endothelial cells
CD45	Leukocytes
CD11b and CD14	Monocytes and macrophages
CD79α & CD19α	B cells
HLA Class II	Antigen presenting cells and lymphocytes
Positive Markers	
CD73/5'-Nucleotidase	Catalyzes production of extracellular adenosine from AMP
CD90/Thy1	Wound repair, cell-cell and cell-matrix interactions
CD105/Endoglin	Vascular homeostasis; modulates TGF-β functions via interaction with TGF-β receptors (RI & RII)

Dominici *et al* 2006

MSC Clinical Applications

- Hematopoietic Stem Cell Transplantation
  - Enhance engraftment
  - Reduce graft versus host disease
- Solid Organ Transplantation
  - Improved graft function
  - Reduced rejection
- Repair Vascular Damage
  - Chemotherapy
  - Radiotherapy
  - Others

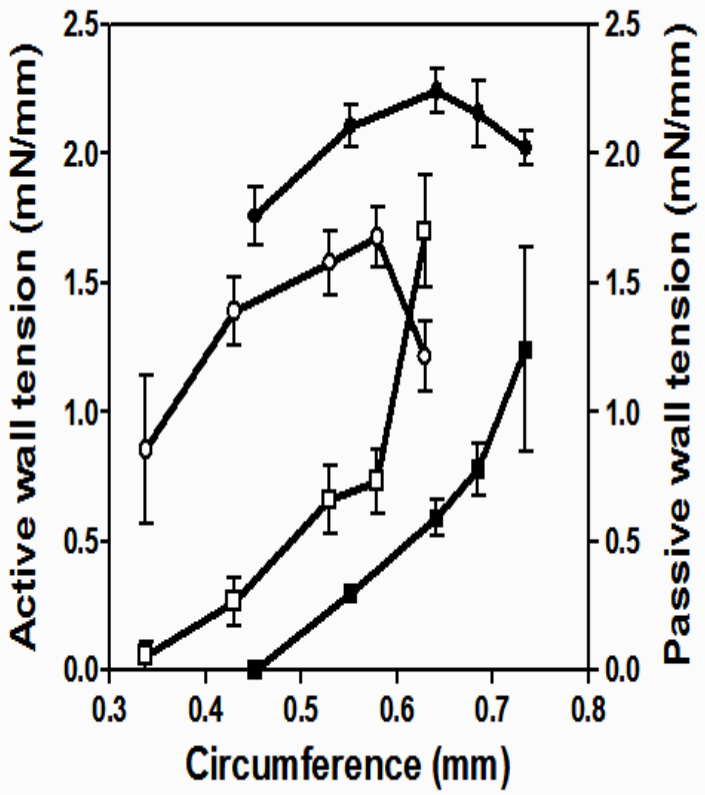
Chemotherapy and Vascular Toxicity

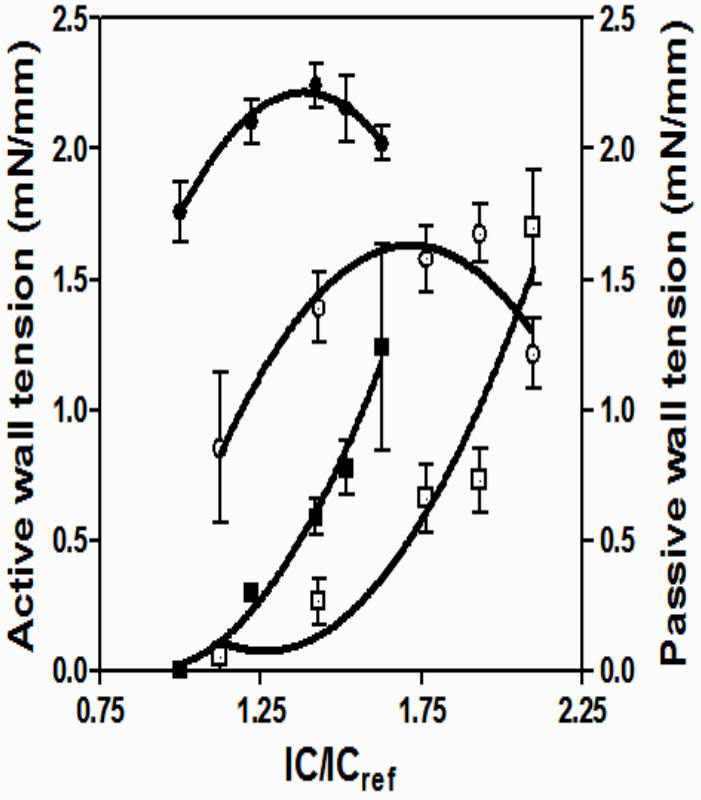
TABLE 1. Cardiotoxicity Profiles of Chemotherapeutic Agents

Drug Class/Name, Generic (Brand)	Cardiac Adverse Events	Relative Frequency of Specific Adverse Effect*	Relative Frequency of Therapeutic Use†	Comment
Anthracyclines/anthraquinolones				
Doxorubicin (Adriamycin) Daunorubicin (Cerubidine) Epirubicin (Ellence, Pharmorubicin) Idarubicin (Idamycin)	CHF/LV dysfunction	+++	+++	Risk of CHF is cumulative dose and schedule dependent; LV dysfunction is secondary to free radical production; increased risk for young/elderly, after mediastinal XRT, female gender, history of cardiac disease; continuous infusion, liposomal delivery systems, or use of dexrazoxane can reduce toxicity; when appropriately administered, incidence of LV dysfunction is <5%
Mitoxantrone (Novantrone)	CHF/LV dysfunction	++	+	Anthraquinone derivative; low propensity for free radical production; myocarditis and arrhythmia can be seen acutely with infusion
Alkylating agents				
Busulfan (Myleran)	Endomyocardial fibrosis Cardiac tamponade	+ +	+	
Cisplatin (Platinol)	Ischemia Hypertension CHF	++ ++++ ++	+++	CHF risk is increased in elderly, after chest XRT, or after prior anthracyclines
Cyclophosphamide (Cytoxan)	Pericarditis/ myocarditis CHF	+ ++	+++	Rare incidence of hemorrhagic myocarditis, more common with high dose CHF risk is increased with cumulative dose, in elderly, after chest XRT, or after prior anthracyclines
Ifosfamide (Ifex)	CHF Arrhythmias	++ ++	++	CHF risk is increased with cumulative dose, prior anthracyclines
Mitomycin (Mutamycin)	CHF	++	+	CHF risk is increased with cumulative dose, prior anthracyclines, chest XRT

Edward *et al* 2004

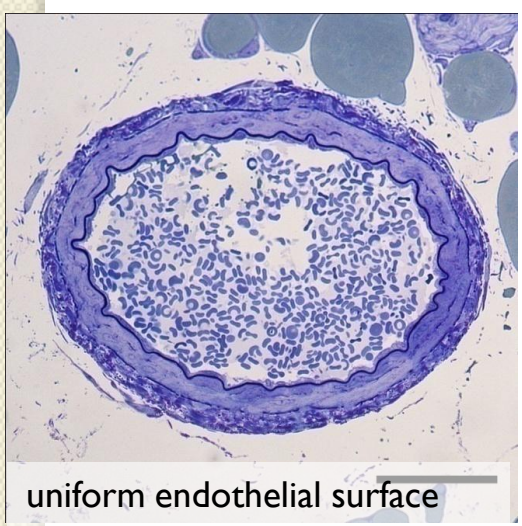
Bu-Cy and Vascular Toxicity

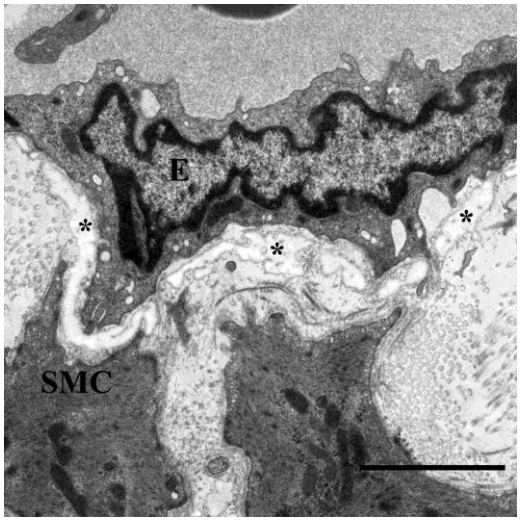




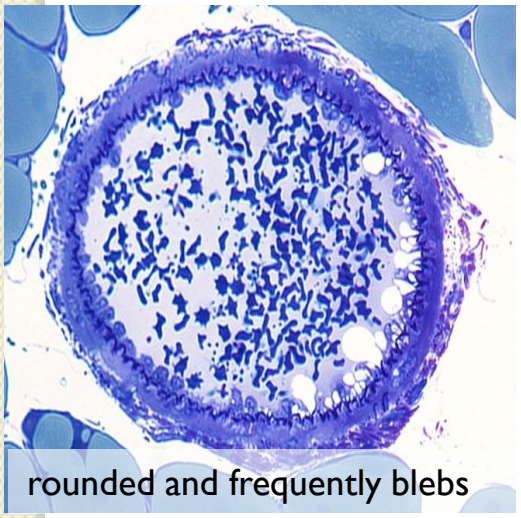
Al-Hashmi *et al*, 2012

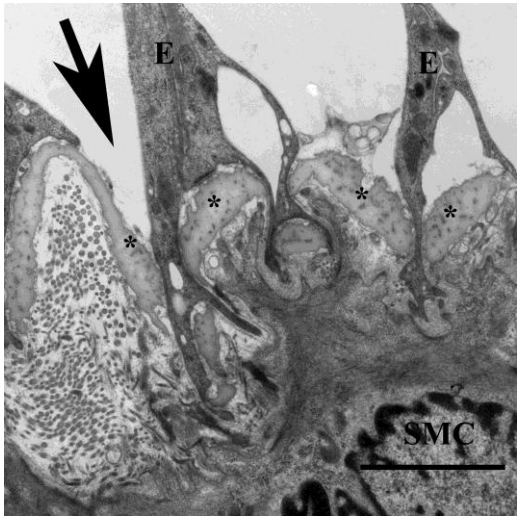
Bu-Cy and Vascular Toxicity


uniform endothelial surface



Control
•Unbroken endothelial cell-cell contact


rounded and frequently blebs



Bu-Cy
•Detaching of endothelial cells
•Cell-cell contacts were disrupted

Al-Hashmi *et al,* 2012

Chemotherapy & Vascular Toxicity

- Cisplatin-based therapy
- Testicular cancer patients
- Before and after chemotherapy

Table 2. Vascular Events

Patient	Age (years)	Event	Time	Platelets* ($\times 10^9$/L)		Fibrinogen* (g/L)		vWF* (%)	
				Before	After	Before	After	Before	After
1	31	MI	Day 15, course 2	393	673	5.0	6.4	170	339
2	37	MI	Day 9, course 1	378	667	4.8	4.4	185	280
3	34	DVT (leg)	At diagnosis	348	264	3.9	3.1	104	139
4	23	DVT (subclavian vein)	Day 22, course 4	239	117	ND		78	95
5	31	DVT (subclavian vein)	Day 9, course 2	217	270	ND		86	140
6	28	PE	Day 11, course 4	285	196	4.6	3.4	88	173
7	22	PE	Day 15, course 2	755	245	7.3	4.4	133	145

MI, myocardial infarction; DVT, deep vein thrombosis; PE, pulmonary embolism; vWF, von Willebrand factor

Nuver *et al*, 2005

MSCs and Vascular Repair

- MSC Source
  - Rat BM
- Injury induction
  - Ligation of proximal left coronary artery
    - MI
- MSC infusion
  - Infarct area (2×10^6)
- Outcomes
  - Expressed muscle and endothelium markers
  - Improved LV function

Dai *et al* 2005

MSCs and Vascular Repair

- MSC Source
  - *Canine BM*
- Injury induction
  - Ameroid constrictor placement
    - Chronic myocardial ischemia
- MSC infusion
  - Intramyocardial injections (10×10^7)
- Outcomes
  - Improved ejection fraction & vascular density
  - Increased vascularity
  - Improved cardiac function
- MSC Differentiation
  - SMCs & ECs

Silva *et al* 2005

MSCs and Vascular Repair

- MSC Source
  - Porcine BM
- Injury induction
  - Occlusion followed by reperfusion of the anterior coronary artery
    - MI
- MSC infusion
  - Intramyocardial injections (20×10^7)
- Outcomes
  - Improved myocardial blood flow
  - Improved ventricular & late cardiac functions
  - Enhanced blood vessels maturation
  - Reduced endomyocardial apoptosis
  - Reduced infarct scarring

Schuleri *et al* 2008

MSCs and Vascular Repair

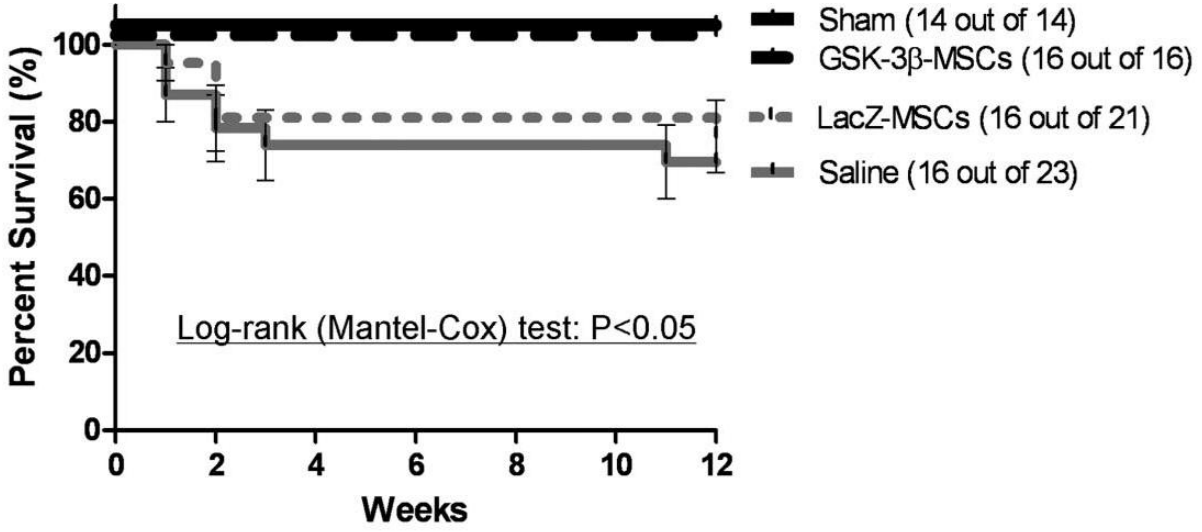
- MSC Source
  - Porcine BM
- Injury induction
  - Carotid artery cannulation
    - MI
- MSC infusion
  - Transendocardial injections (10×10^7)
- Outcomes
  - Differentiated into cardiomyocytes and vascular structures
  - Stimulated endogenous cardiac stem cells proliferation and differentiation
  - Stimulated cardiomyocyte replication
  - Reduced infarct size

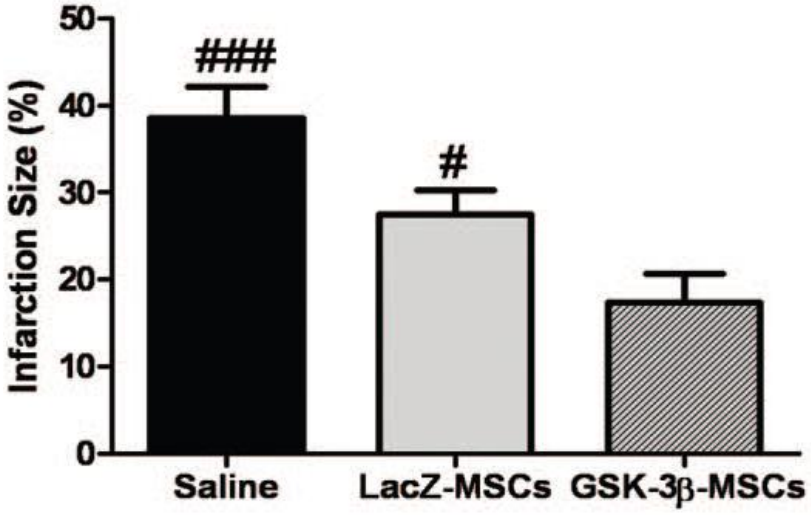
Hatzistergos *et al* 2010

MSCs and Vascular Repair

- MSC Source
  - C57Bl/6 mice BM
    - Express GSK-3 β
- Injury induction
  - Carotid artery ligation
    - MI
- MSC infusion
  - Border zone of MI ($1.5x\ 10^5$)
- Outcomes
  - GSK-3β increased MSCs survival
  - GSK-3 β -MSCs induced cardiomyocyte differentiation and angiogenesis
  - GSK-3 β–MSCs increased capillary density
  - GSK-3 β–MSCs upregulated paracrine factors (VEGF-A)

Cho *et al* 2011



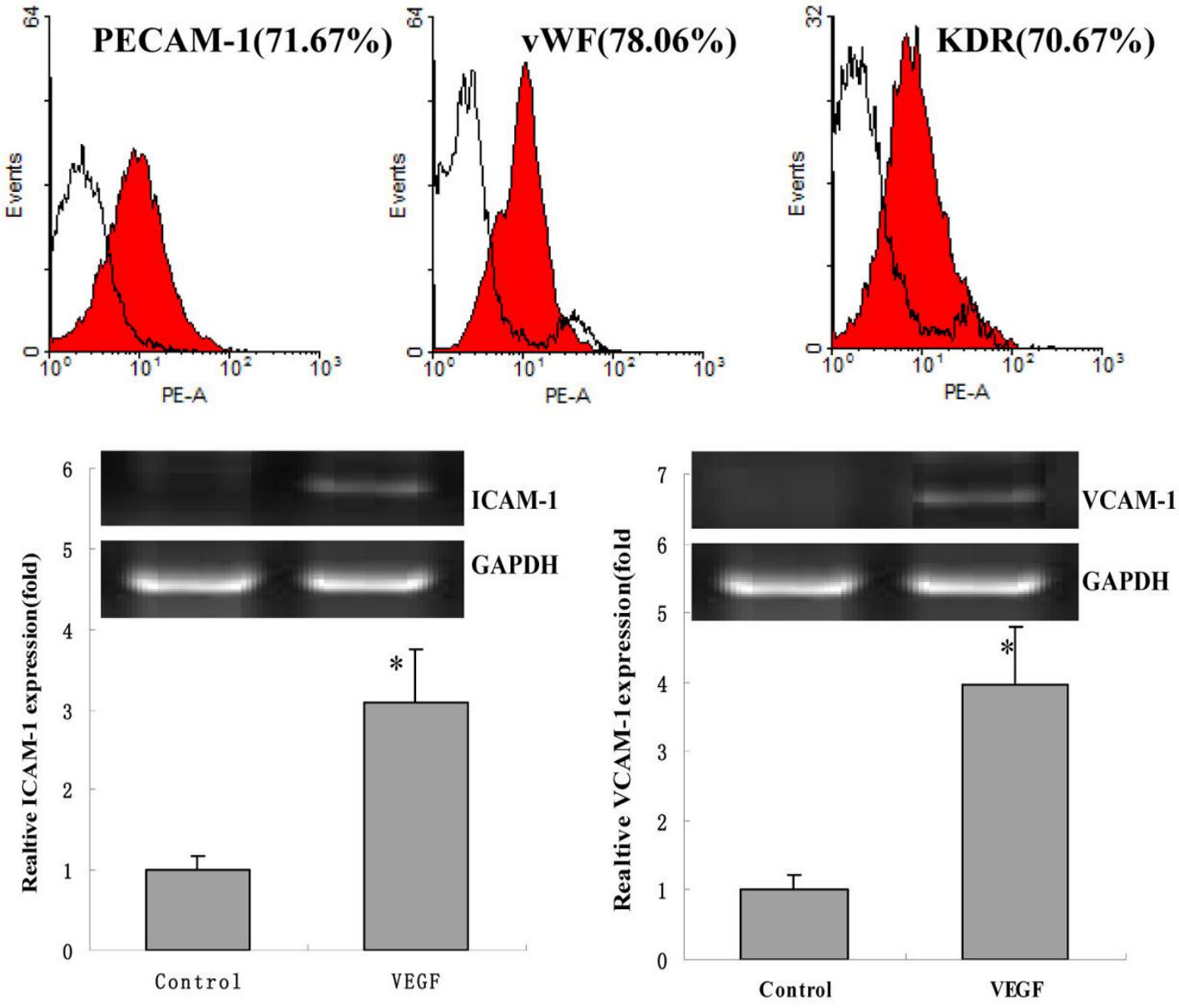


Cho *et al* 2011

MSCs and Vascular Repair

- MSC Source
  - C57Bl/6 mice BM
- Injury induction
  - Removal of endothelium with a flexible wire
    - Carotid artery injury
- MSC infusion
  - IV injection (1×10^5)
- Outcomes
  - Differentiated into neo-ECs in the injury
  - Contributed to vascular remodeling
  - Expressed different markers
  - Formed the tube-like structure

Li *et al* 2013


PECAM-1(71.67%)
vWF(78.06%)
KDR(70.67%)
Events
PE-A
ICAM-1
GAPDH
VCAM-1
Realtive ICAM-1 expression(fold)
Realtive VCAM-1expression(fold
Control
VEGF
*

Li *et al* 2013

MSCs in clinical practice (Meta Analysis)

- Searched databases
  - PubMed, OVID, EMBASE, the Cochrane Library, and ClinicalTrials.gov
- Outcome
  - Improved LVEF in patients
- Efficacy of Cells in transplantation influenced by
  - Source of cells
  - Route of infusion
  - Type of injury
- BMCs & MSCs infusion is a safe and effective therapy to improve vascular repair

Xiao *et al* 2014; Xu *et al* 2014; Tian *et al* 2014

Precautions

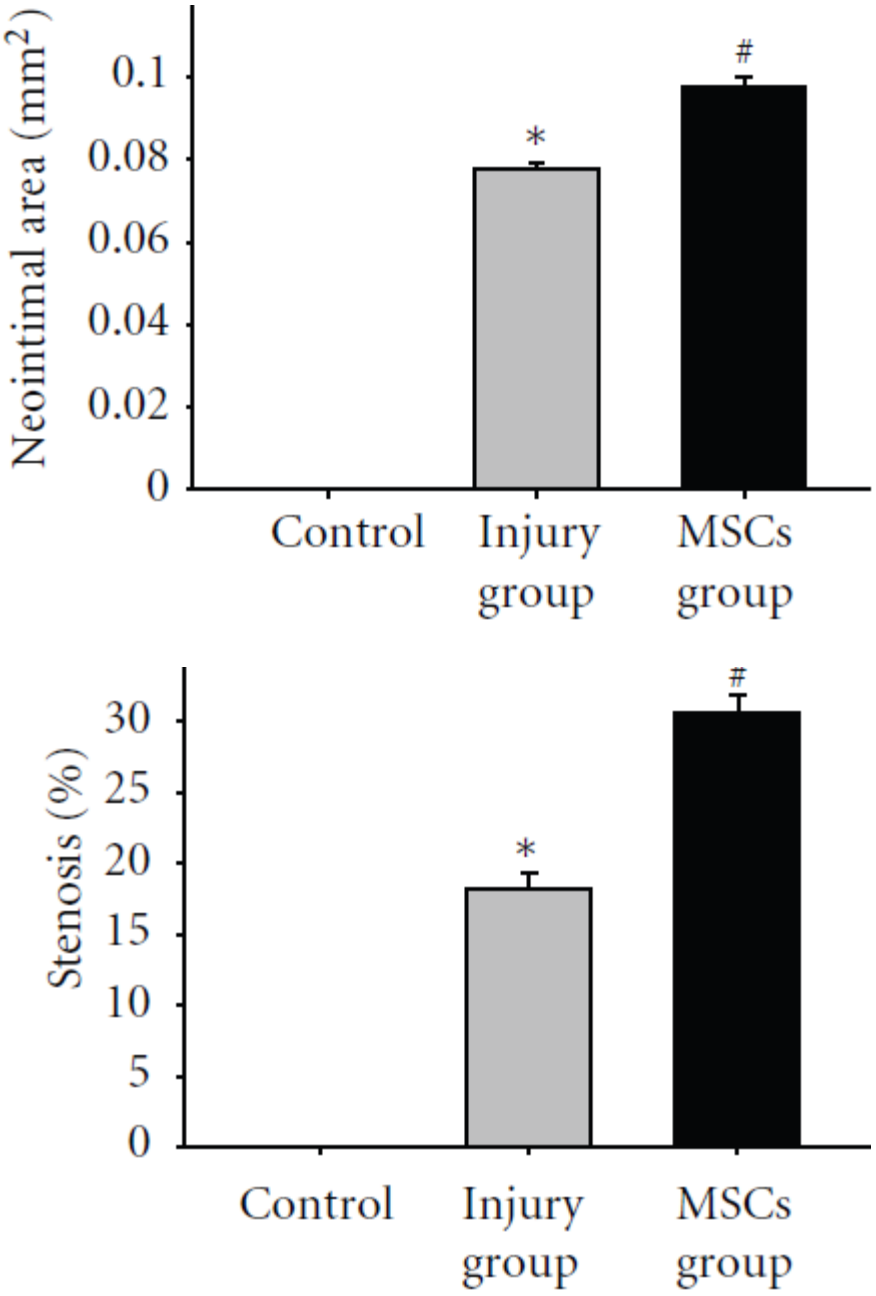
- Intra-myocardial calcification
- Vascular calcification
- Increase aortic stiffness
- Systolic hypertension
- Transient improvement
- Unregulated differentiation

Liao *et al* 2012; Dai *et al* 2005

Prospective

- MSC therapy under intensive investigation
  - Establish clinical relevant vascular injury models
  - Sources & doses
  - Timing
  - Protocols
- Collaboration
  - Type of disease
  - Data sharing


THANKS



Liao *et al* 2012

MSCs in clinical practice

Table 3: Ongoing clinical trials on MSCs: condition, intervention/dose, and followup in patients around the world (http://www.clinicaltrials.gov).

World	Condition	Intervention	Time frame	Phase/Status
Florida (USA)	Chronic ischemic LV dysfunction secondary to MI	10 and 20 intramyocardial injections of 2 million MSCs (low dose) or 20 million (high dose)/0.25–0.5 cm^3 for a total of 20 million or 200 million cells, respectively	6–18 months	Phase I/II (unknown)
	Chronic ischemic LV dysfunction and heart failure secondary to MI	Transendocardial injection of autologous human cells (bone marrow or mesenchymal). 40 million cells/mL delivered in either a dose of 0.25 mL/injection for a total of 100 million × 10 injections or a dose of 0.5 mL/injection for a total of 200 million × 10 injections	6–18 months	Phase I/II (unknown)
	Chronic ischemic LV dysfunction secondary to MI	Transendocardial injection of autologous versus allogeneic MSCs. 40 million cells/mL delivered in either a dose of 0.5 mL/injection × 1 injection for a total of 20 million, a dose of 0.5 mL/injection × 5 injections for a total of 100 million, or a dose of 0.5 mL/injection × 10 injections for a total of 200 million MSCs	6–13 months	Phase I/II (active)
	Nonischemic dilated cardiomyopathy	Transendocardial injection of autologous versus allogeneic MSCs. 20 million cells/mL delivered in a dose of 0.5 mL/injection × 10 injections for a total of 100 million of MSCs	6–12 months	Phase I/II (active)
Maryland (USA)	Chronic ischemic LV dysfunction secondary to MI	10 and 20 intramyocardial injections of 2 million MSCs (low dose) or 20 million (high dose)/0.25–0.5 cm^3 for a total of 20 million or 200 million of autologous human MSCs, respectively	6–18 months	Phase I/II (unknown)
	Chronic ischemic LV dysfunction secondary to MI	Transendocardial injection of autologous versus allogeneic MSCs. 40 million cells/mL delivered in either a dose of 0.5 mL/injection × 1 injection for a total of 20 million, a dose of 0.5 mL/injection × 5 injections for a total of 100 million, or a dose of 0.5 mL/injection × 10 injections for a total of 200 million MSCs	6–13 months	Phase I/II (active)
France (Europe)	Chronic myocardial ischemia; LV dysfunction	Transendocardial intramyocardial injections of 60 million autologous MSCs	30 days–2 years	Phase I/II (active)
China (East Asia)	ST-elevation MI	Intracoronary human umbilical WJ-MSC transfer	4 months–1 year	Phase II (active)
Korea (East Asia)	Acute MI	Intracoronary injection of single dose of autologous bone-marrow-derived MSCs (1 million) cells/kg	6 months	Phase II (completed)
India (South Asia)	ST-elevation acute MI	A Single Dose of Intravenous infusion of Allogenic MSCs	6 months	Phase I/II (active)

Elnakish *et al* 2012

Table 2. Basic criteria for defining human MSCs

Cultivation	*Adherence to plastic in standard culture conditions*	
Phenotype	*Positive expression (⩾95%)*	*Negative expression (⩽2%)*
	CD 73	CD 14 or CD 11b
	CD 90	CD 19 or CD 79α
	CD 105	CD 34
		CD 45
		HLA-DR
In vitro differentiation:	Under specific stimulus, cells will differentiate into osteoblasts, adipocytes, and chondroblasts	

Cancer Gene Therapy (2014), 12 – 23