Characterization of MSCs for Clinical Applications

M-CERSI Workshop May 8, 2013 Baltimore

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ISCT Cytotherapy (2006) Vol. 8, No. 4, 315–317 Taylor & Francis

POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

M Dominici[1], K Le Blanc[2], I Mueller[3], I Slaper-Cortenbach[4], FC Marini[5], DS Krause[6], RJ Deans[7], A Keating[8], DJ Prockop[9] and EM Horwitz[10]

Conflict of Interest Statement

I have no financial relationships to disclose within the past 12 months relevant to my presentation

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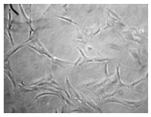
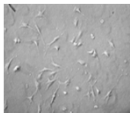
Isolation and Identification

- Markers (mouse and human):
  - Positive: CD105 (endoglin), CD73 (membrane-bound ecto-5'-nucleotidase) and CD44 (hyaluronate receptor)
  - Negative: CD45 (hematopoietic marker) and CD31(endothelial marker)
- Morphology

Human

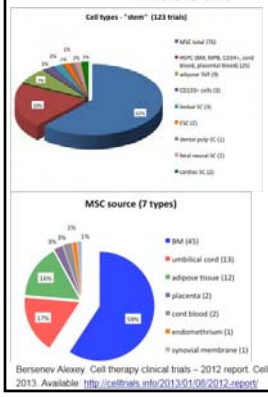


Mouse



Cell therapy clinical trials – 2012 report

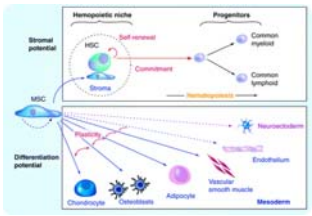
by ALEXEY BERSENEV on JANUARY 8, 2013 · 6 COMMENTS
in ANNUAL REPORTS



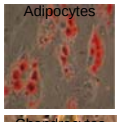
Category	Conditions	Clinical phase
Heart disease	Myocardial infarct and heart failure	Phase I and II
Immune diseases	Multiple sclerosis	Phase I and II
	Graft-versus-host disease	Phase I, II and III
	Crohn's disease	Phase II and III
	Organ transplantation and organ failure	Phase I and II
	Diabetes Mellitus	Phase I and II
	Lupus	Phase I and II
Bone and cartilage defect	Bone and cartilage fracture, Osteogenesis imperfecta and degenerative diseases	Phase I, II and III
Others	Miscellaneous	Phase I, II and III

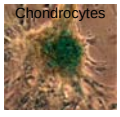
Bersenev Alexey. Cell therapy clinical trials – 2012 report. CellTrials.blog. January 8, 2013. Available: http://celltrials.info/2013/01/08/2012-report/

Differentiation



Regenerative Med. (2006) 1(4), 497–509



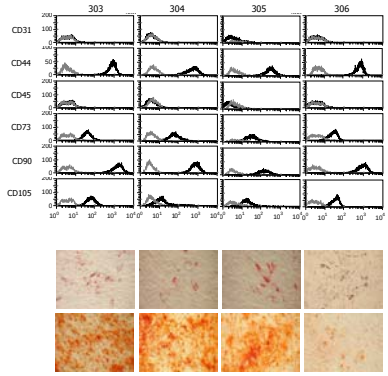


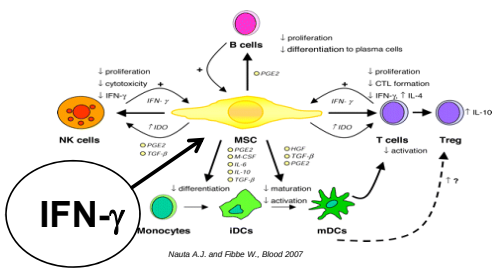
Are the ISCT criteria for MSC predictive of immune suppressive function?

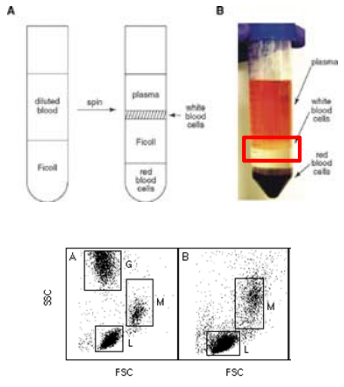
Table1. Human MSC donor descriptions

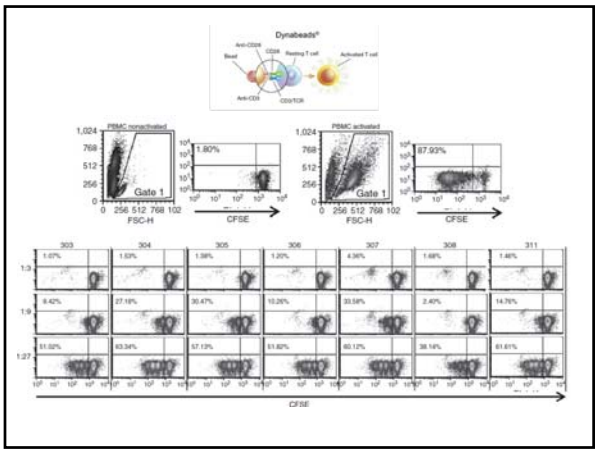
Donors #	Gender	Age (years)
303	Female	72
304	Female	68
305	Female	62
306	Female	62
307	Female	78
308	Male	50
311	Female	70

Interrogating the functional immune plasticity of MSCs as deployed by cytokine licensing

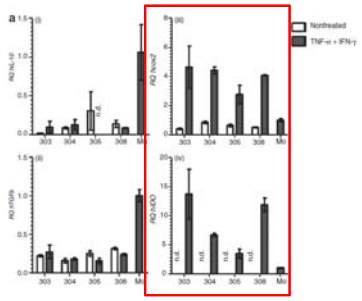




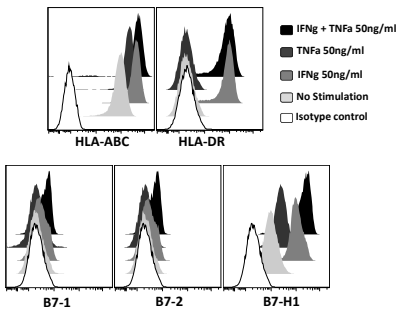




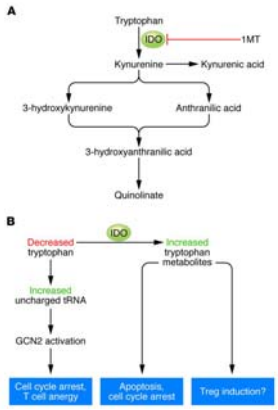
MSC Transcriptional induction by IFN-γ



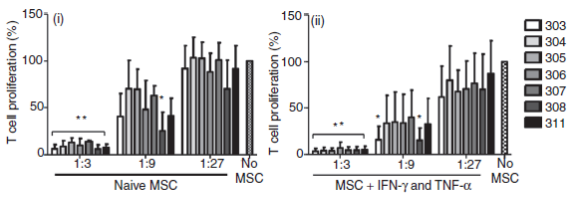
Upregulation of MHC on human MSCs by IFNg and TNFa stimulation



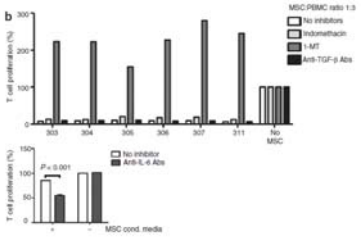
72 Hrs stimulation

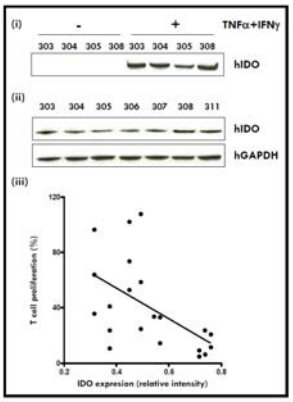


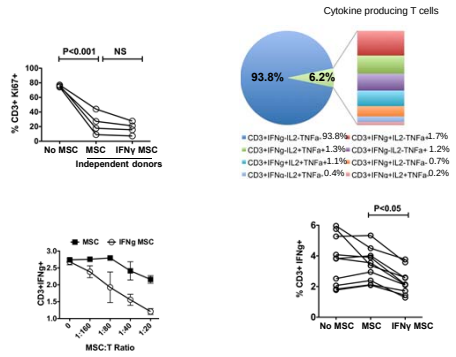
MSC suppression intensity varies amongst subjects and is uniformly enhanced by IFN-γ



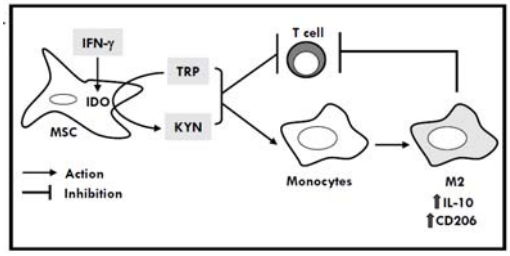
IDO inhibition aborts suppression by MSC.
COX2 and TGFβ blockade have no effect
IL6 driven exuberance is unleashed







	IFN alpha	IFN gamma	TNFalpha	Variegation
MHCI	+	+	+	-
MHCII	-	+++	-	-
B7H1	-	++	-	-
CCL2	++	++	-	++
CCL5	-	-	++	-
CCL7	-	++	+	+
CCL8	-	++	+	++
IL1beta	-	-	++	+
IL6	-	-	++	+
IL8	-	-	++	-
IDO	++	++++	+	++
MMP3	-	-	+++	-
IL10	-	-	-	-

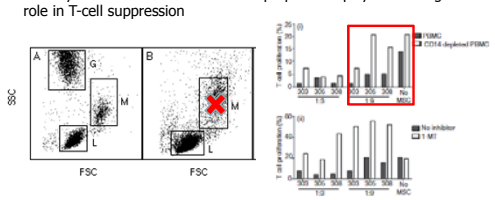


Human MSC Suppression Correlates With Cytokine Induction of Indoleamine 2,3-Dioxygenase and Bystander M2 Macrophage Differentiation

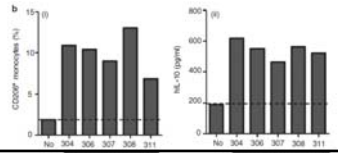
Moïra François[1,2], Raphaëlle Romieu-Mourez[2], Mengyang Li[2] and Jacques Galipeau[2,4]

Molecular Therapy. 2012 Jan;20(1):187-95.

Monocytes contained with a "PBMC" preparation play a meaningful role in T-cell suppression



MSC instruct monocytes to adopt an M2 suppressor phenotype



Take home points

- A "classic" ISCT analysis is great for identity but uninformative for MSC immune veto function
- Cytokine [Interferon-γ +/- TNFα] licensing deploys an immune plasticity phenotype which correlates form to function
- MSC veto function varies in human population and correlates with IDO upregulation by IFNγ
- MSCs deploy a multifaceted response to INFγ in addition to IDO which may be correlated to function as well [e.g. B7-H1 and others]
- Reductionist interrogation of MSCs *per se* is less susceptible to intra and inter-observer variability than classic MLR and variants

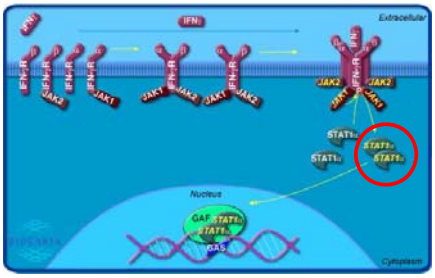
MSC are like Sushi

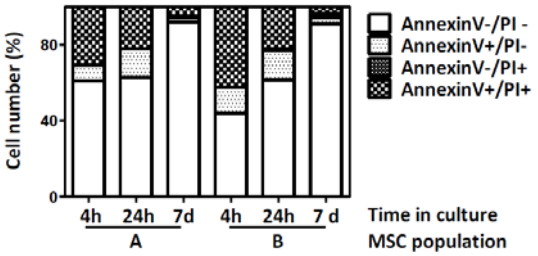
Fresh is best

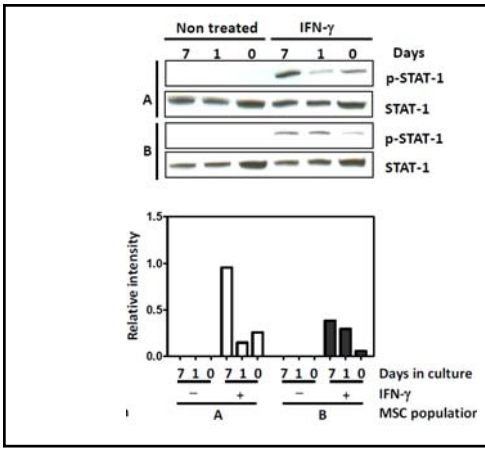
Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-γ licensing

MOÏRA FRANÇOIS[1,2], IAN B. COPLAND[3], SHALA YUAN[2], RAPHAËLLE ROMIEU-MOUREZ[2], EDMUND K. WALLER[3] & JACQUES GALIPEAU[2,3,4]

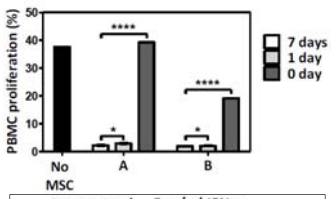
Cytotherapy. 2012 Feb;14(2):147-52. Epub 2011 Oct 27.

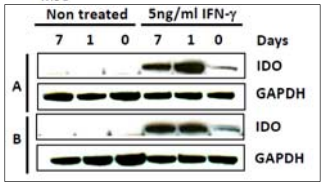


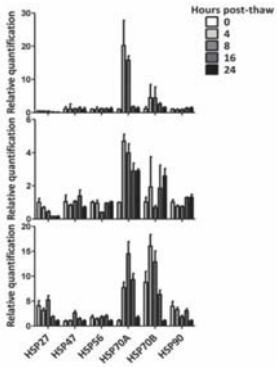




Cryopreserved MSCs have a markedly impaired IFNγ response







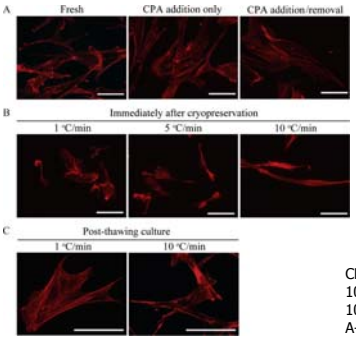
What about homing in vivo?

The Freezer burn effect

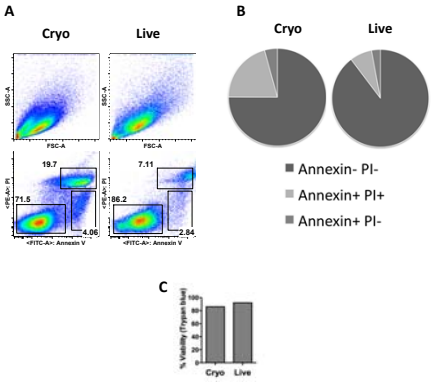
Effects of osmotic and cold shock on adherent human mesenchymal stem cells during cryopreservation

Xia Xu[a,*], Yang Liu[b], Zhanfeng Cui[c], Yuping Wei[a], Liang Zhang[a]

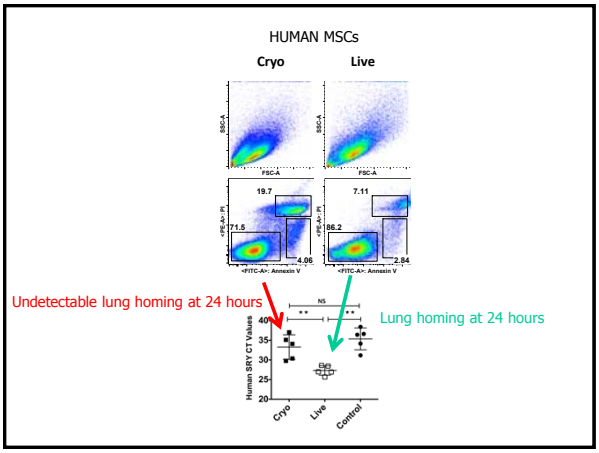
Journal of Biotechnology 162 (2012) 224–231



CPA:
10%DMSO
10% FBS
A-MEM



Conclusion



Cytotherapy, 2013; 0: 1–8

International Society for Cellular Therapy ISCT

Immunological characterization of multipotent mesenchymal stromal cells—The International Society for Cellular Therapy (ISCT) working proposal

MAURO KRAMPERA[1,*], JACQUES GALIPEAU[2,*], YUFANG SHI[3], KARIN TARTE[4,5] & LUC SENSEBE[4,6], ON BEHALF OF THE MSC COMMITTEE OF THE INTERNATIONAL SOCIETY FOR CELLULAR THERAPY (ISCT)

Table I. Suggestions for the assessment of regulatory properties of human MSCs

1. A standard immune plasticity assay should be implemented with IFN-$\gamma \pm$ TNF-α used as model *in vitro* priming agent.
2. Functional analysis of an expanded cell product may provide mechanistic insights on intra-study and inter-study variance in clinical response among patients.
3. The use of purified responders would be widely practicable and should provide more generalizable guidance on relative functional potency of MSCs and as a companion to clinical trials.
4. Interrogating the IDO response as part of an *in vitro* licensing assay should be considered central.
5. Conclusions drawn on the basis of xenorecipient animal models on how to conduct clinical trials should be drawn with caution.
6. The prospective hypothesis-driven analysis of lymphocyte populations in patient groups treated with MSC should be encouraged.
7. Clinical analysis should also include the monitoring of whether injected MSCs are the target of an immune response.

Merci!

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