XV Congresso SIdEM
Torino, 9-12 novembre 2011

Cellule Staminali: dalla biologia alla clinica

Cellule Staminali e tessuto adiposo

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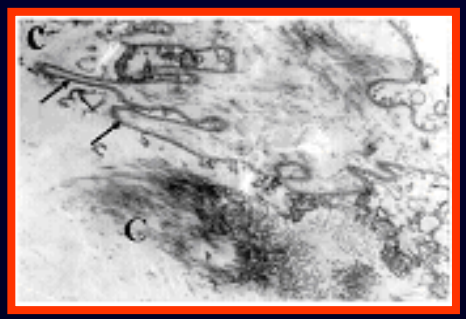
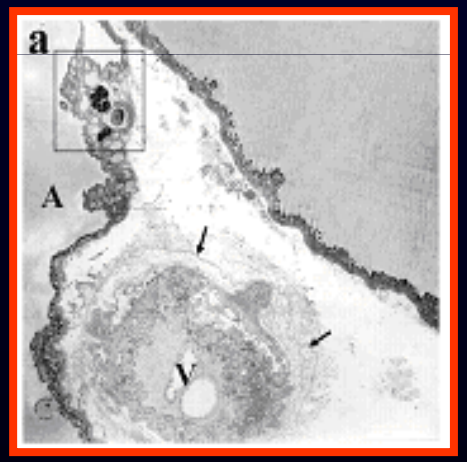
Adipose tissue in plastic surgery
- Radiotherapy side effects -

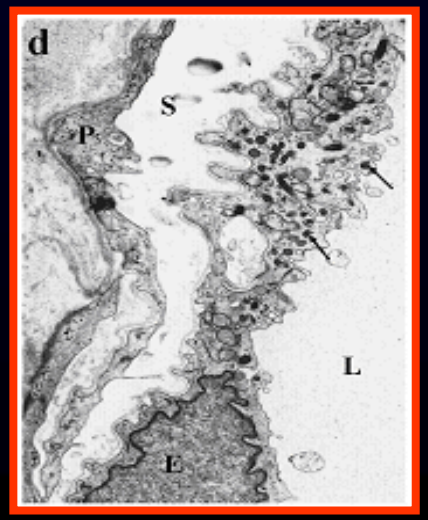
Reduction of capillary vessels density



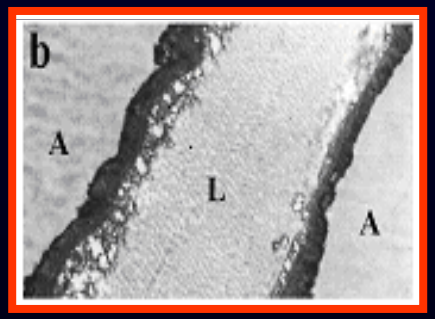
Hyper-permeability



Edema



Fibrosis



By courtesy of G. Rigotti and A. Sbarbati, Verona

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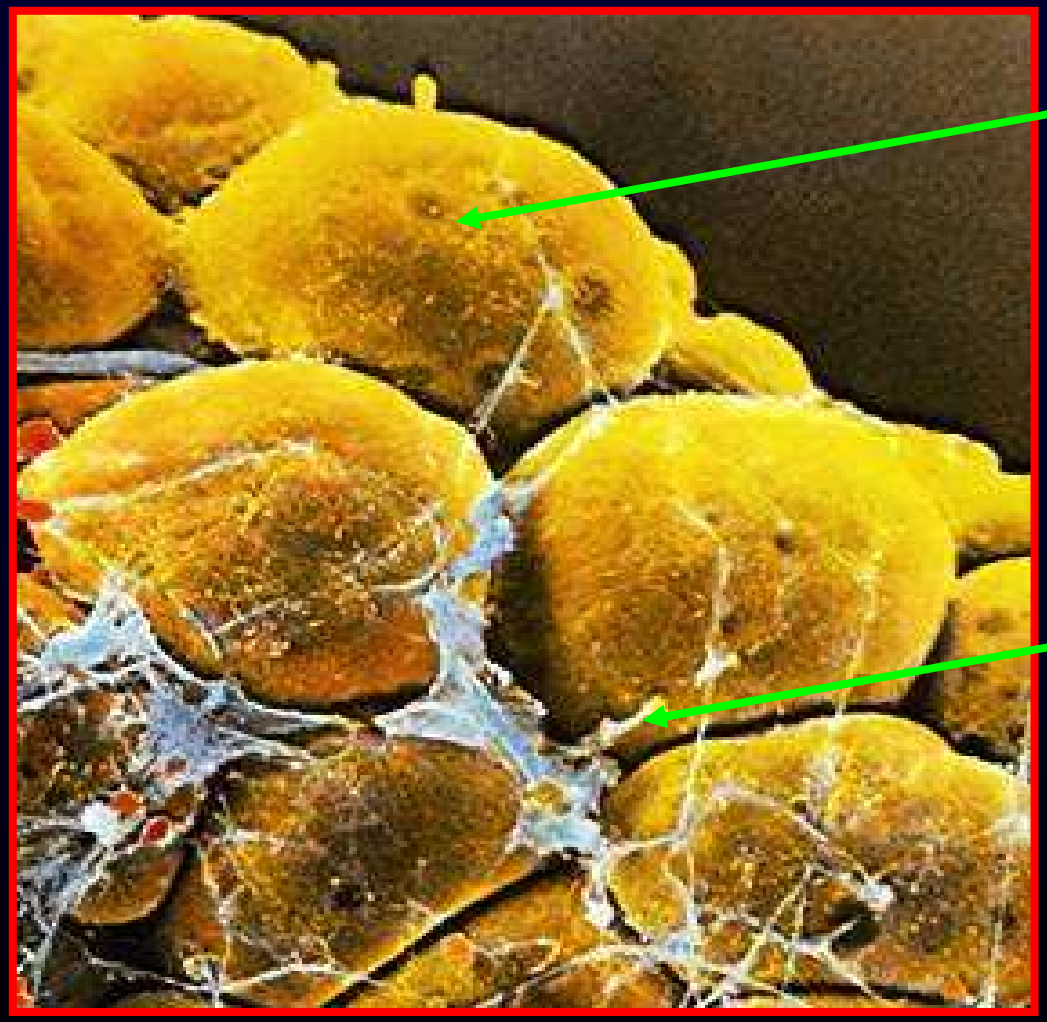
Adipose tissue



Adipocytes/pre-adipocytes
(70-90%)

Stromal - Vascular
Fraction
(10-30%)

- Endothelial cells
- smooth muscle cells
- monocyte/macrophages
- “fibroblasts”

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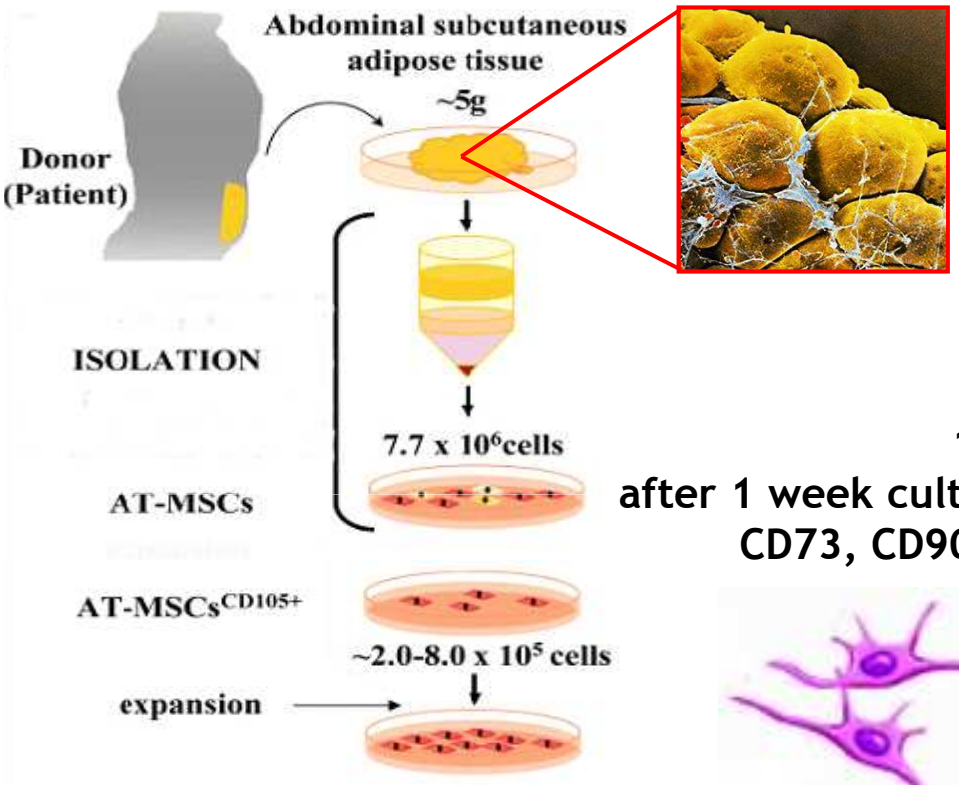
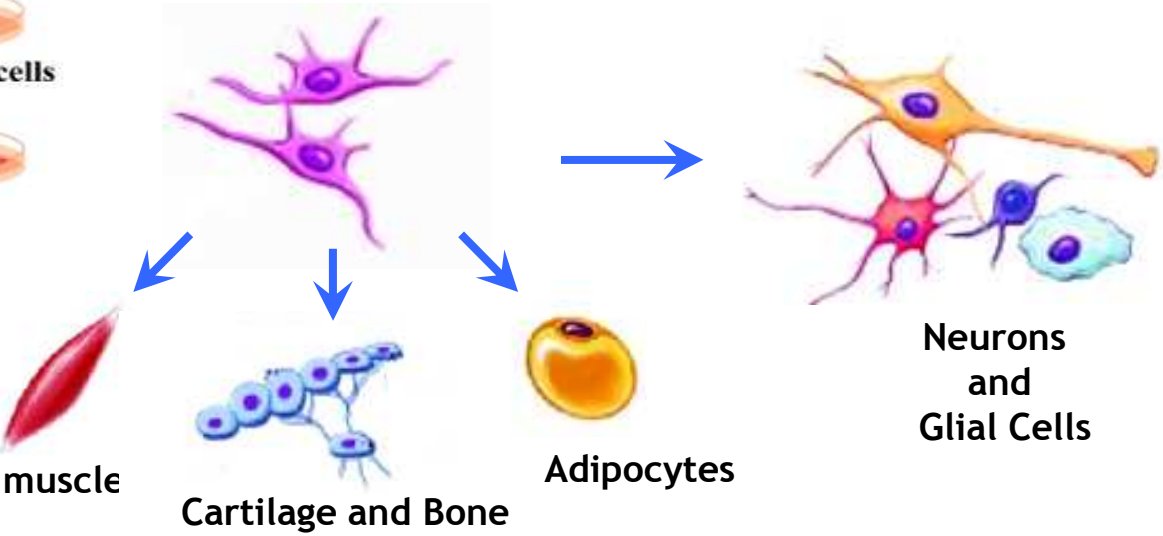
Collection of SVF by means of lipoaspirate



Adipose tissue is a highly specialized connective tissue composed by adipocytes (70-90%) and Stromal-Vascular Fraction (muscle cells, endothelial cells, blood cells, preadipocytes and fibroblasts)

1-2% of SVF,
after 1 week culture cells selectively express CD73, CD90 and CD105 (AT-MSCs)



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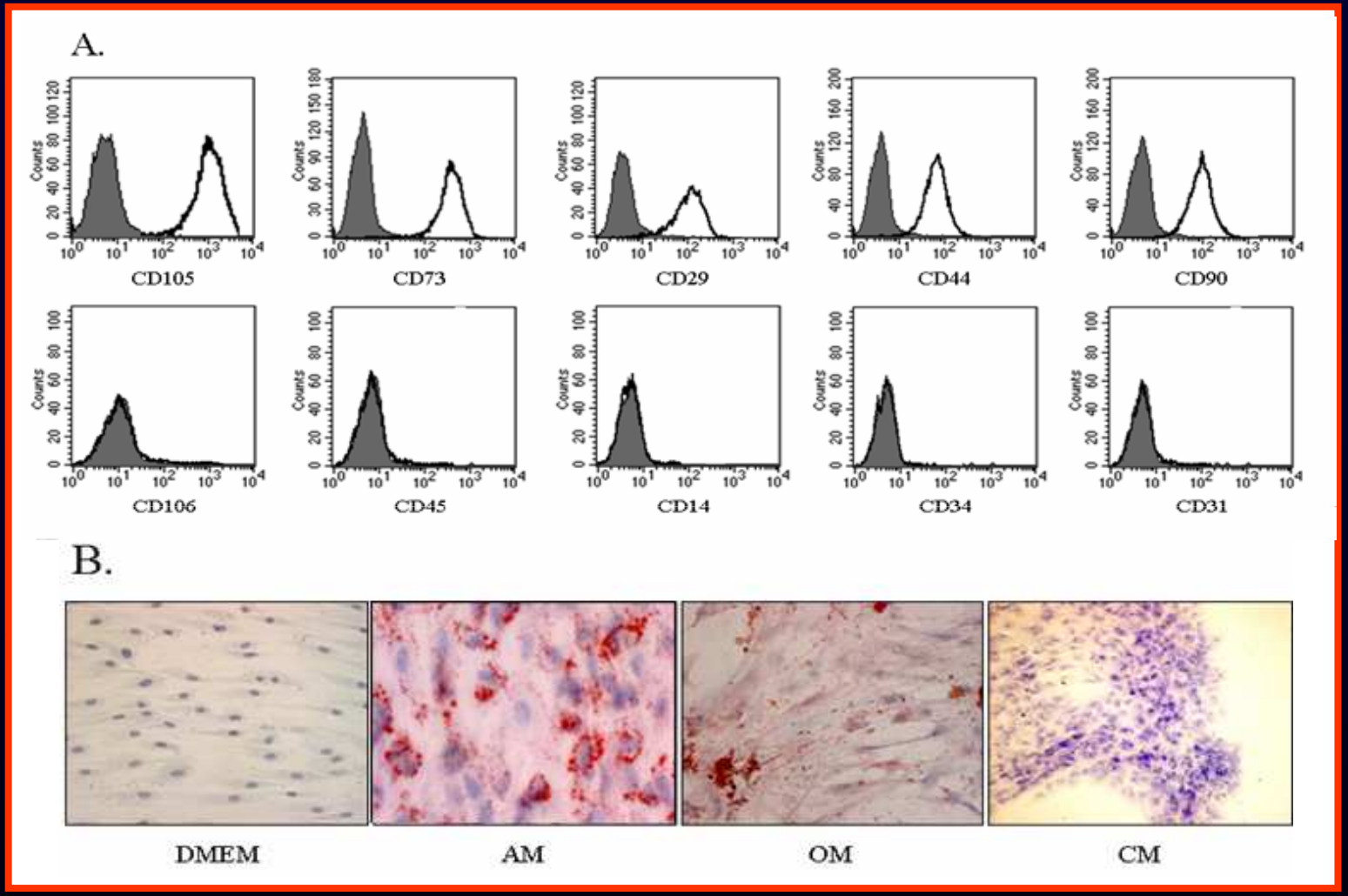
Adipose tissue-derived MSC (AT-MSC)



G. Rigotti, A. Marchi, M. Galiè, G. Baroni, D. Benati, M. Krampera, A. Pasini, A. Sbarbati. Clinical treatment of radiotherapy tissue damages by lipoaspirates transplant: a healing process mediated by adipose-derived adult stem cells (ASCs). *Plast Reconstr Surg*, 2007; 119:1409-1422.

F. Mosna, L. Sensebé, M. Krampera. Human bone-marrow and adipose tissue mesenchymal stem cells: a user's guide. Stem Cells and Development 2010;19:1449-70.

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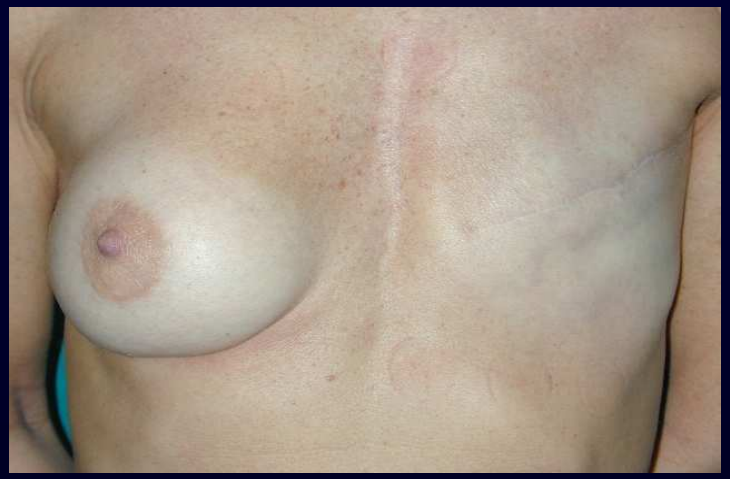
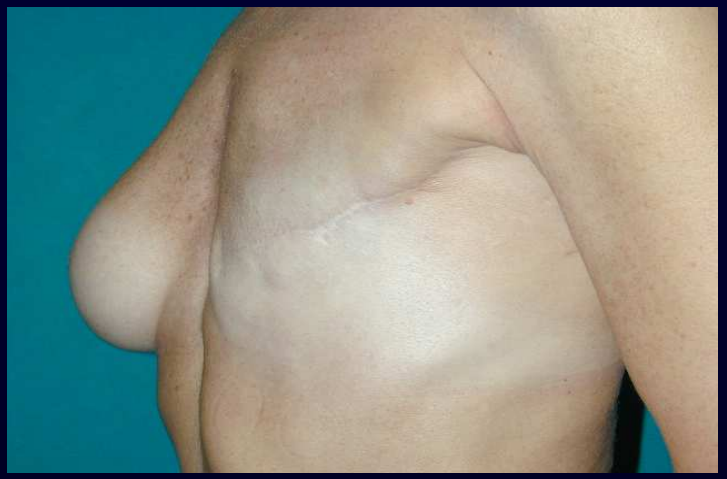
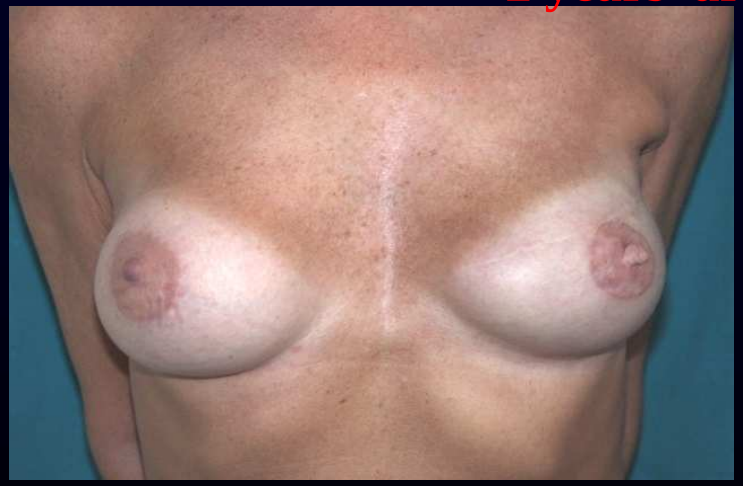
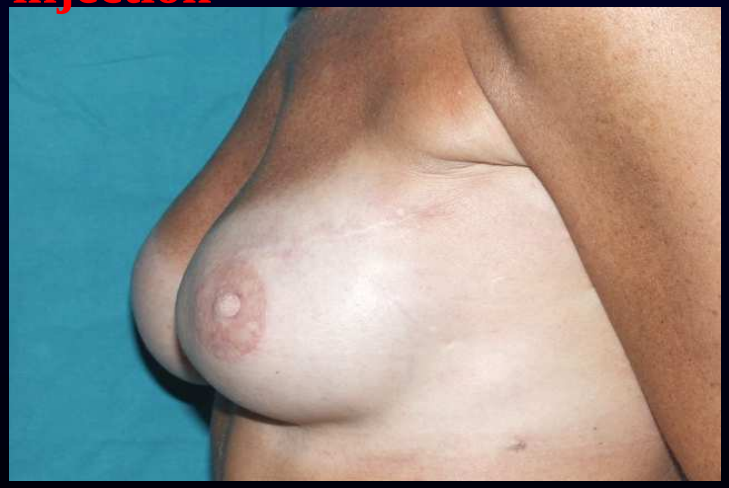
SVF subcutaneous injection
- Clinical effects -





2 years after injection





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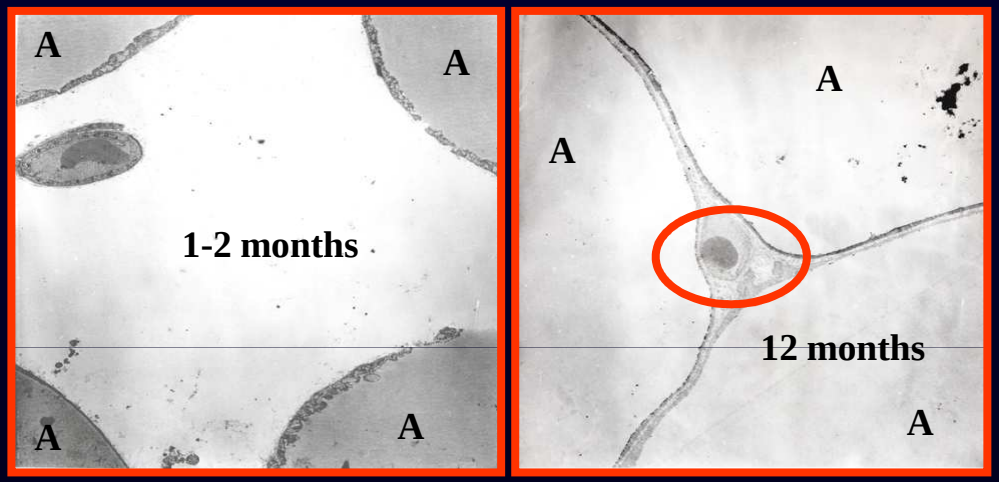
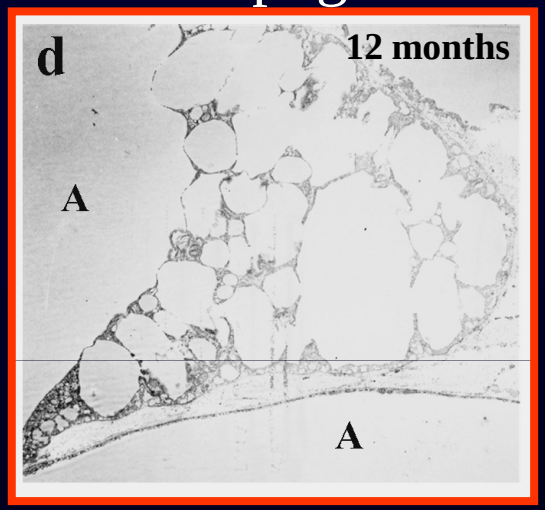
SVF subcutaneous injection
- Microscopical effects -

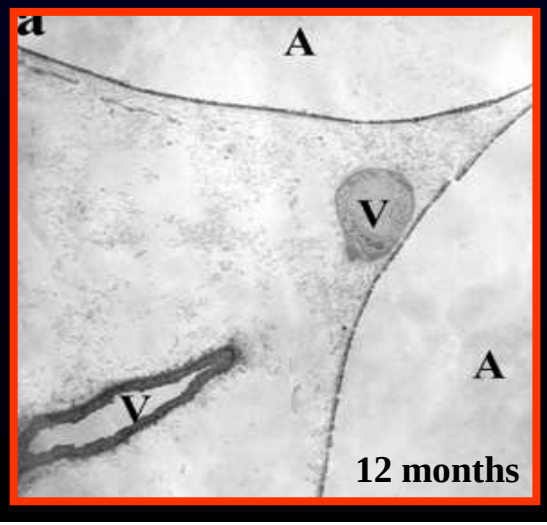
Edema reduction



Neo-adipogenesis



Reduction of fibrosis
Neo-vascularization
Increase of capillary density



By courtesy of G. Rigotti and A. Sbarbati, Verona

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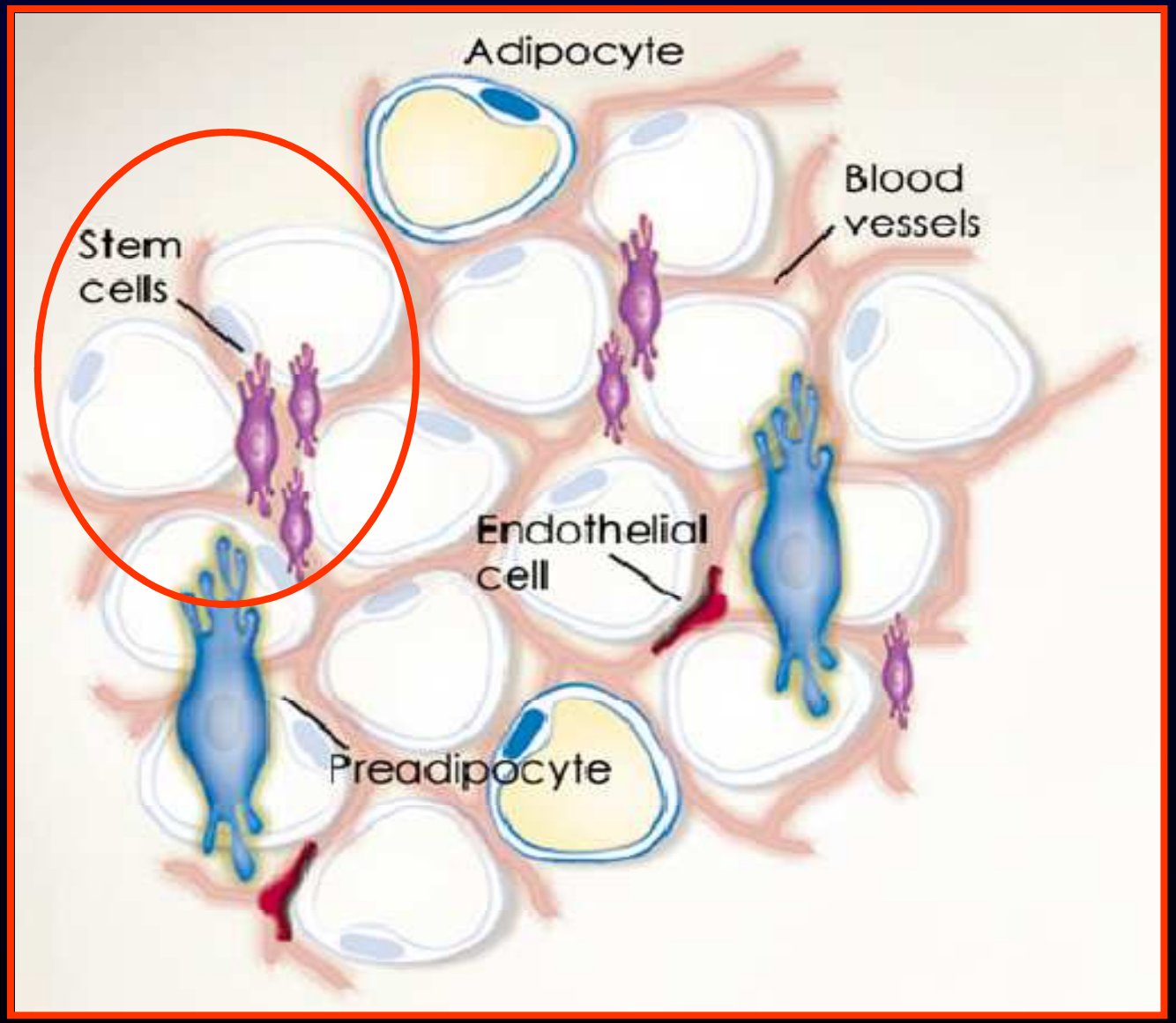
Adipose tissue



B. Lindroos, R. Suurinen, S. Miettinen. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev and Rep*, 7:269-291. 2011.

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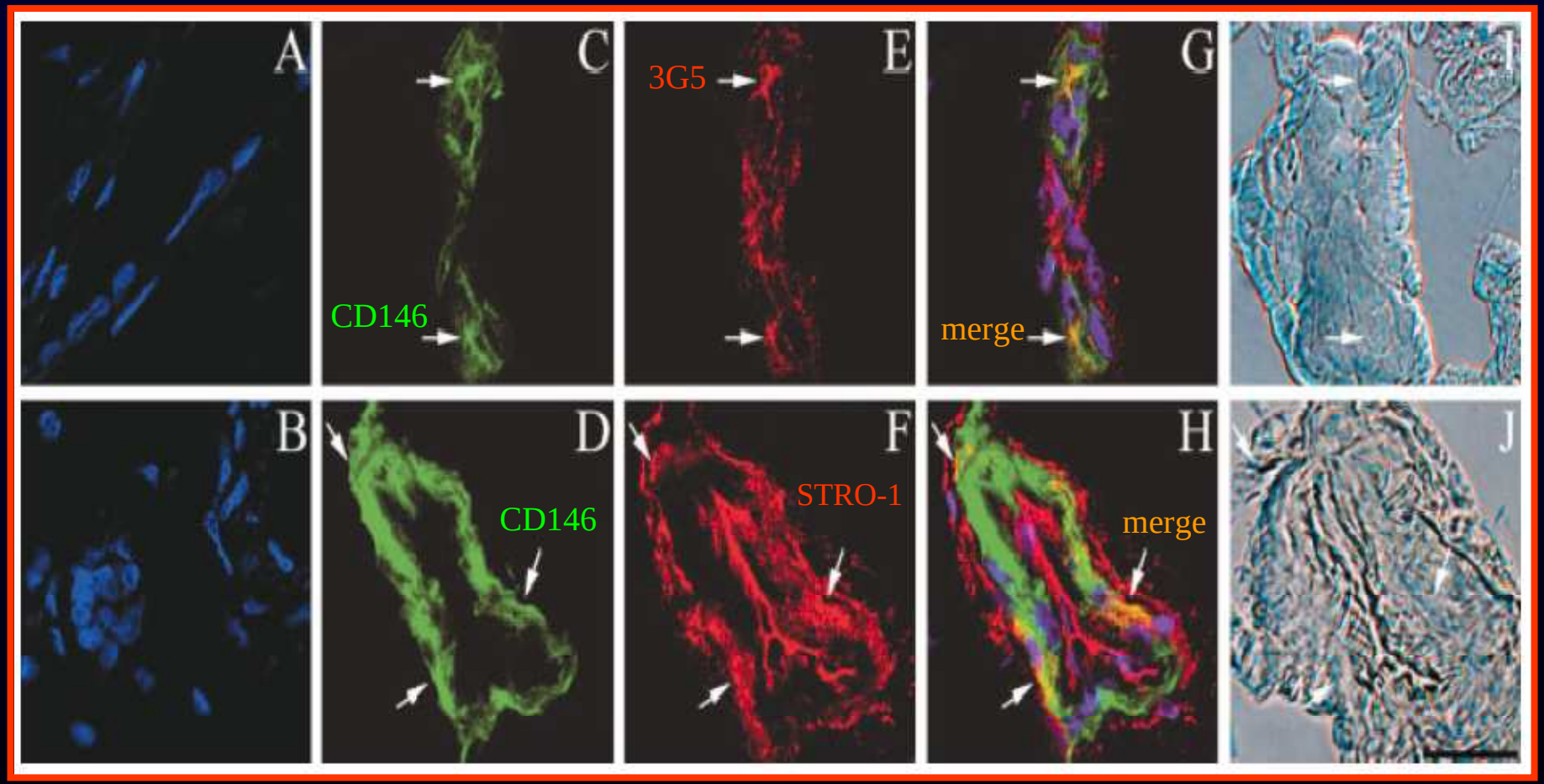
Adipose tissue-derived MSC (AT-MSC) - *in vivo* perivascular localization -



Zanettino ACW, et al.. Multipotential Human Adipose-Derived Stromal Stem Cells Exhibit a Perivascular Phenotype In Vitro and In Vivo. *J Cell Physiol* 2008; 214: 413–421.

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Adipose tissue-derived MSC (AT-MSC)

surgical resection

tumescent lipoaspiration

ultrasound-assisted lipoaspiration

sterile **microdissection**
~ 1 cm³ fat lobules

washing with sterile PBS

collagenase digestion
30-90 min., 37 °C under permanent shaking
(Krebs-Ringer solution (pH 7.4) buffered with 25 mM Hepes (20 mg/ml BSA) + 1.5 mg/ml collagenase)

filtration
(100 µm nylon mesh)

centrifugation
(1,200 g, 10 min.)

removing erythrocytes
(erythrocyte lysis buffer pH 7.3)

washing with sterile PBS

expansion medium
(DMEM-LG + 10 FBS + 1% antibiotic/antimycotic solution)
plating the cells at a density of 150,000 / cm²

separation of cells at 60 – 70 % confluence
plating the cells at 4,000/cm² for ~ 15 passages

lineage-specific differentiation protocols

adipose tissue | muscle | bone | cartilage | other tissues

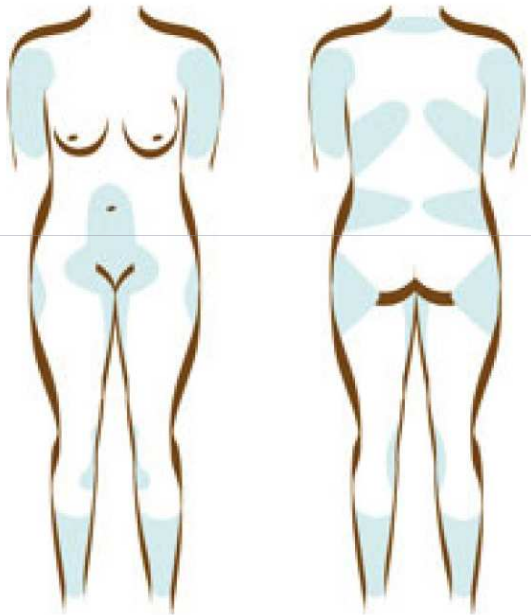

Figure 1. Anatomical regions most commonly associated with liposuction (http://www.aestheticsurgery.ie/images/liposuction1.jpg).

A. Schaffler, C. Buchler. Concise Review: Adipose Tissue-Derived Stromal Cells – Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* 2007; 25:818-827.

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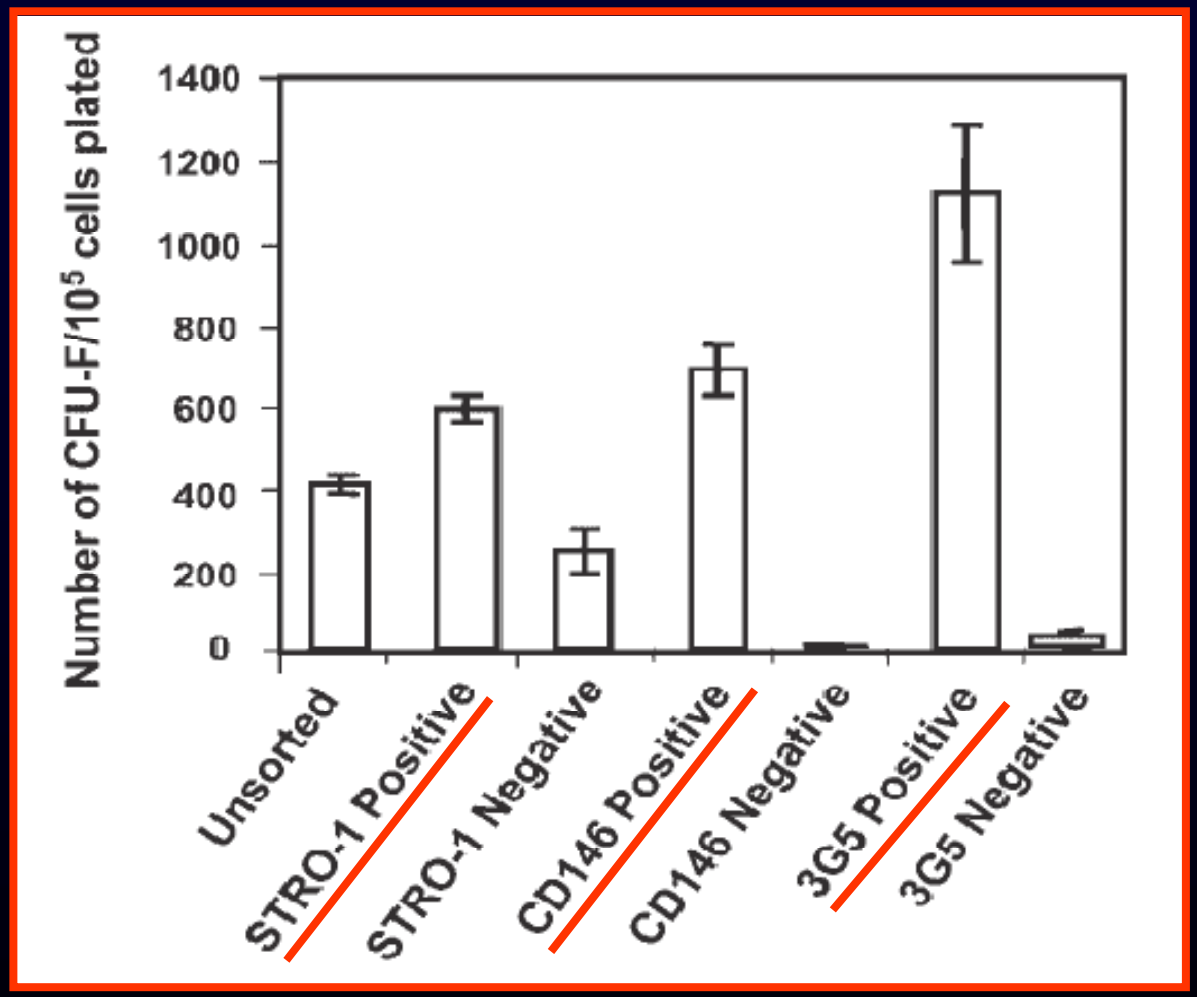
Adipose tissue-derived MSC (AT-MSC)
- *in vitro* clonogenicity -



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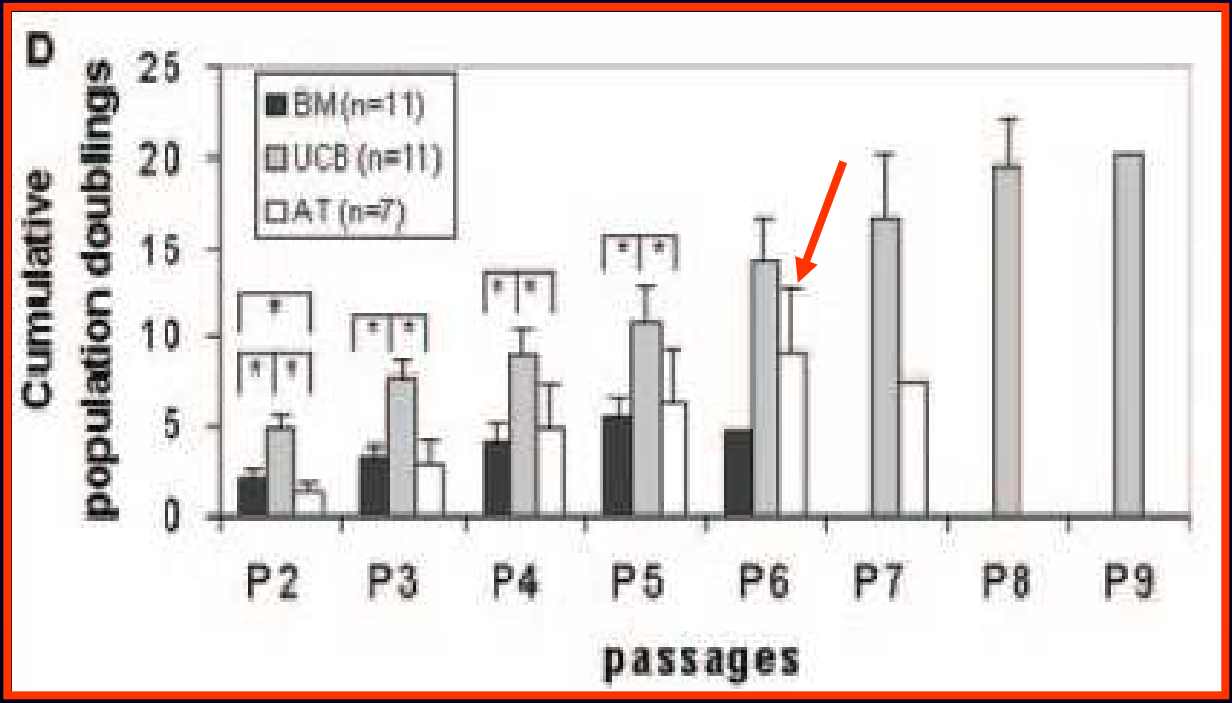
Adipose tissue-derived MSC (AT-MSC)
- *in vitro* expansion and senescence -



S. Kern, H. Eichler, J. Stoeve, H. Kluter, K. Bieback.. Comparative analysis of MSCs from bone marrow, umbelical cord blood, or adipose tissue. *Stem Cells* 2006; 24: 1294–1301.

	Senescence ratio up to passage 2	Maximal passage
BM (n = 21)	23.6%	P 7 (9.5%)
UCB (n = 26)	34.6%	>P 10[a] (3.9%)
AT (n = 18)	5.6%	P 8 (5.6%)
	p = .02[b]	

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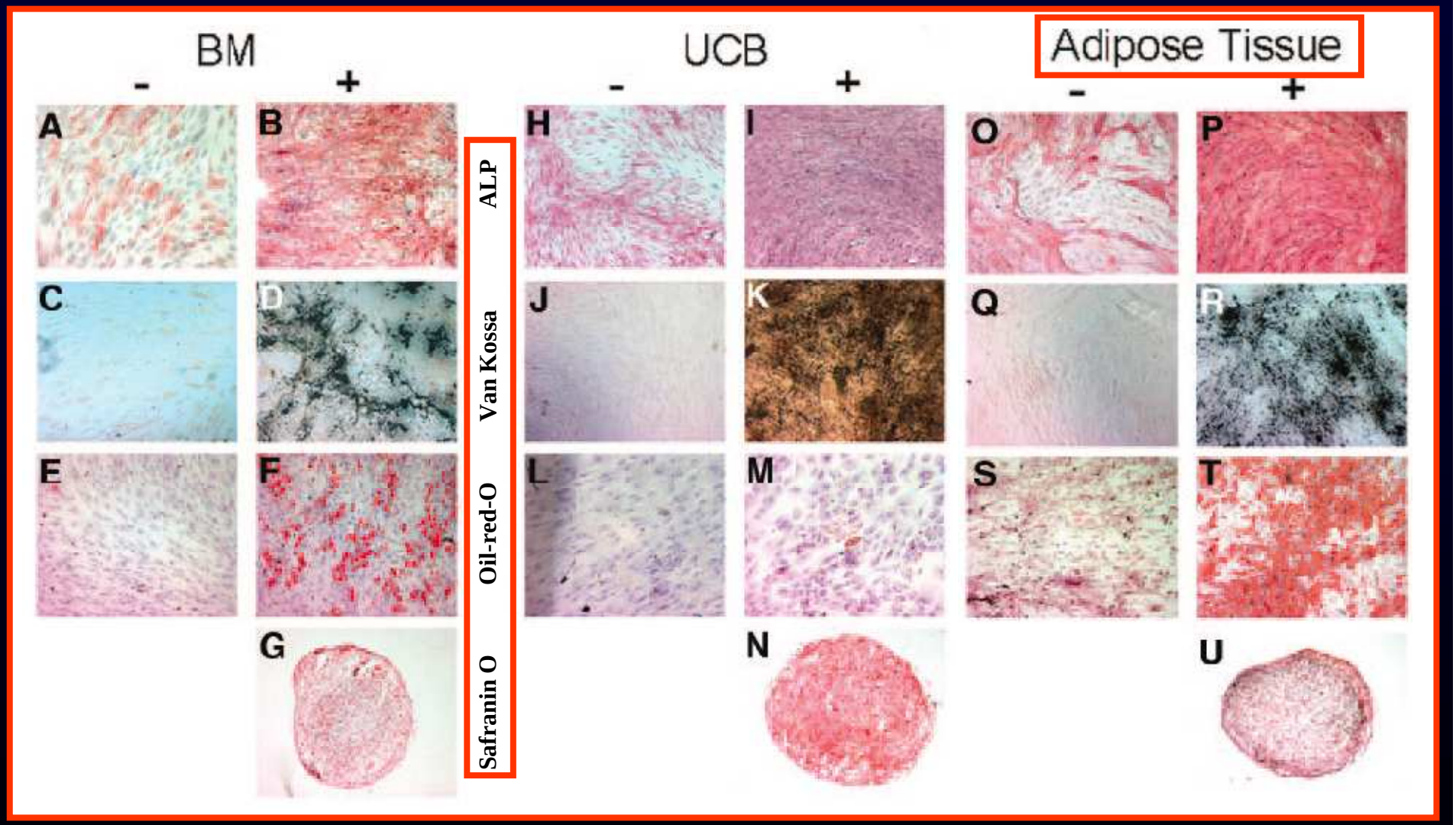
Adipose tissue-derived MSC (AT-MSC)
- *in vitro* differentiation -



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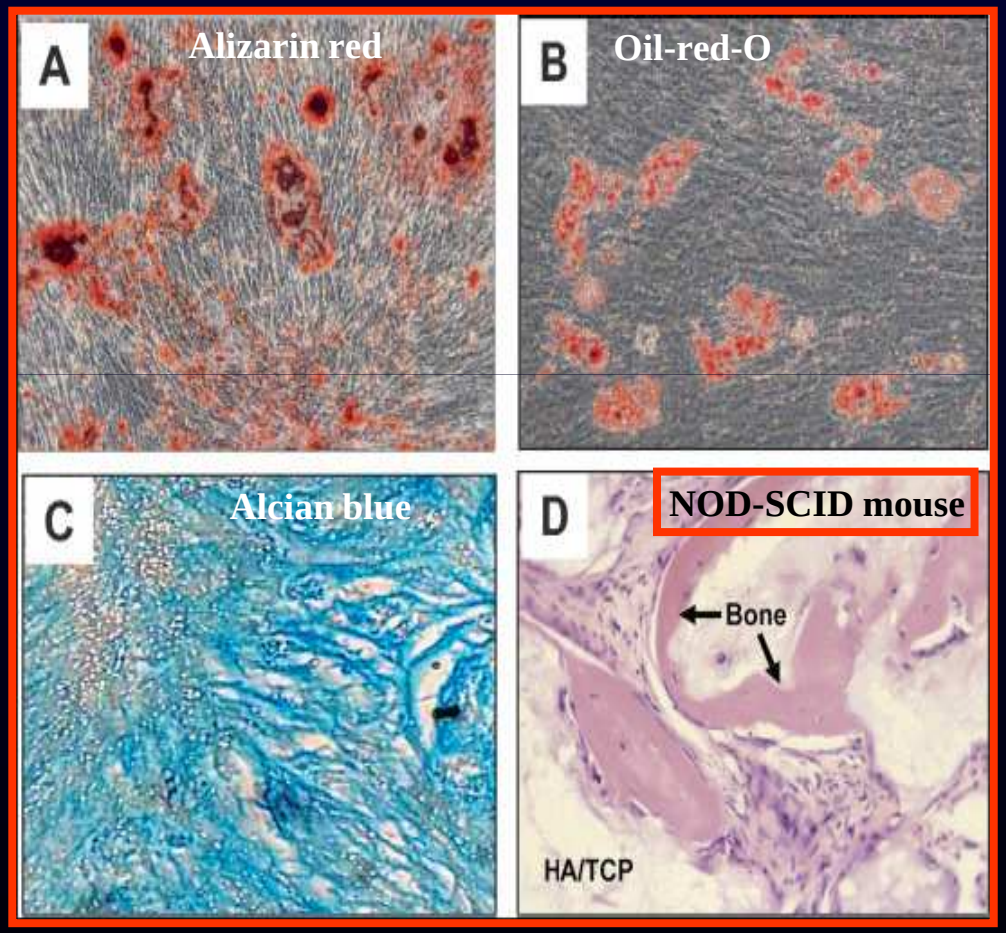
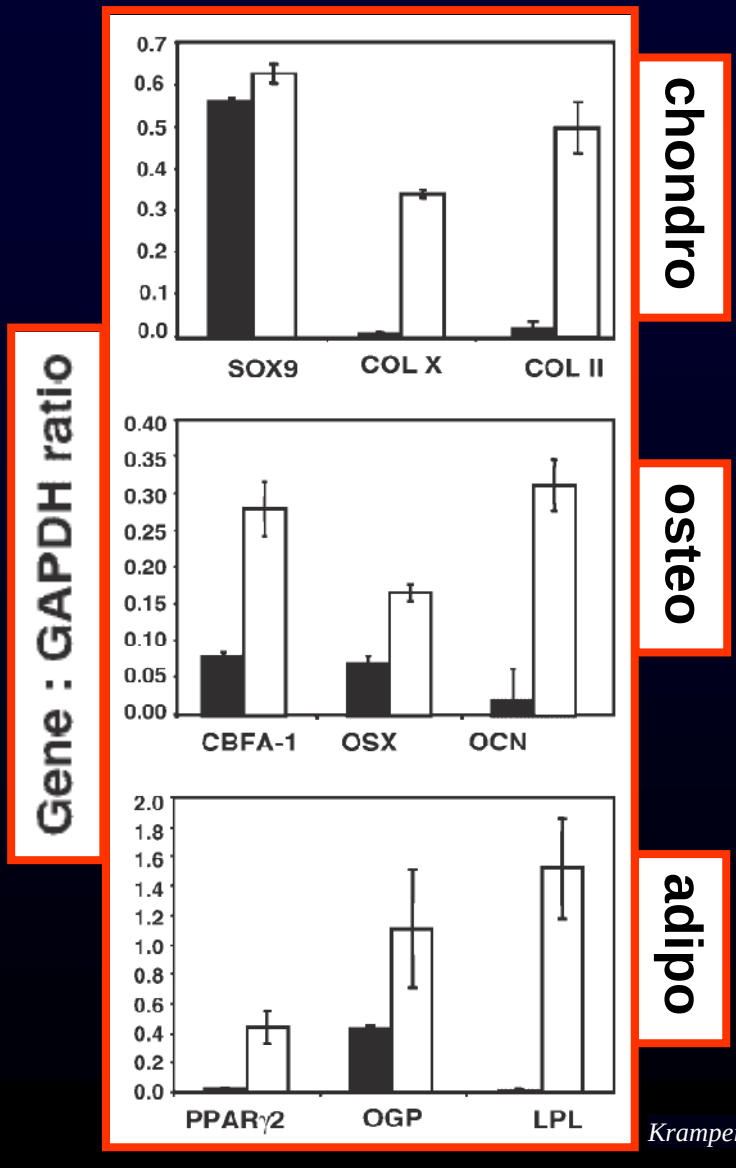
Adipose tissue-derived MSC (AT-MSC)
- *in vitro* and *in vivo* differentiation -

35G^{+} AT-MSC





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Adipose tissue-derived MSC (AT-MSC)

ATMSC-positive cellular markers and genes	ATMSC-negative cellular markers and genes
CD9	**CD11b**
CD10	**CD14**
CD13	**CD19**
CD29	CD31
CD44	**CD34**
CD49 (d)	**CD45**
CD49 (e)	**CD79α**
CD54	CD80
CD55	CD117
CD59	CD133
CD73	CD144
CD90	**HLA-DR**
CD105	**c-kit**
CD106	MyD88
CD146	STRO-1
CD166	Lin
HLA I	HLA II
Fibronectin	
Endomucin	
ASMA	
Vimentin	
Collagen-1	

IFATS criteria

(International Federation of Adipose Therapeutics and Science)
5th Annual Scientific Meeting,
Indianapolis, Indiana, USA
October 2007

IFATS Collection

Stem Cells 2008;26

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Adipose tissue-derived MSC (AT-MSC)

Antibody	BM (%) (n = 9)	UCB (%) (n = 10)	AT (%) (n = 9)
CD44	97.5 ± 5.1	99.7 ± 0.5	99.8 ± 0.2
CD73	90.0 ± 20.0	99.3 ± 1.3	99.6 ± 0.5
CD90	99.1 ± 2.5	97.8 ± 7.1	99.9 ± 0.2
CD14	1.2 ± 1.1	0.8 ± 0.9	2.4 ± 5.0
CD34	1.6 ± 1.4	1.2 ± 1.5	5.0 ± 5.1
CD45	5.2 ± 3.7	3.8 ± 3.6	3.8 ± 5.1
CD105[a]	88.1 ± 7.4	72.4 ± 20.0	90.4 ± 5.9
CD133	1.3 ± 1.1	2.9 ± 5.5	2.9 ± 3.5
CD29	99.0 ± 1.5	99.8 ± 0.4	99.5 ± 1.3
HLA I	95.2 ± 6.0	94.3 ± 6.8	98.8 ± 2.8
CD106[b]	66.3 ± 22.7	70.0 ± 23.6	30.3±18.6
HLA II	4.2 ± 6.1	0.8 ± 1.2	4.4 ± 6.2
CD144	4.5 ± 9.3	2.4 ± 4.0	2.4 ± 2.5

The table shows mean values of the percentage of positive cells ± standard deviation to the total number of cells analyzed.
[a]Significant differences were observed between UCB compared with BM and AT ($p < .05$).
[b]Significant differences were observed between AT compared with BM and UCB ($p < .05$).
Abbreviations: AT, adipose tissue; BM, bone marrow; UCB, umbilical cord blood.

S. Kern, H. Eichler, J. Stoeve, H. Kluter, K. Bieback.. Comparative analysis of MSCs from bone marrow, umbelical cord blood, or adipose tissue. *Stem Cells* 2006; 24: 1294–1301.

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Culture-related AT-MSC variability

Antigen	Surface marker expression					
	FBS		HS		SF/XF	
CD9	+	[75, 98, 219]	+	[98, 219]	+	[98]
CD10	++	[75, 98, 219]	++	[98, 219]	++	[98]
CD13	+++	[75, 85, 98, 150, 219]	+++	[98, 219]	+++	[98]
CD14	±	[75, 84, 147, 220, 221], *	++	[147], *		
CD19	±	*	±	*		
CD29	+++	[73, 75, 85, 98, 147, 219–221]	+++	[98, 147, 219]	+++	[98]
CD31	+/±	[75, 85, 147, 172, 219, 221, 222]	±	[98, 147, 172, 219]	±	[98, 172]
CD34	+	[75, 85, 147, 150, 172, 219–221]	+	[98, 99, 172, 219]	+	[98, 99, 172]
CD44	+++/++	[75, 85, 147, 219–222]	++	[98, 147, 219]	+++	[98]
CD45	+/±	[75, 147, 150, 219–222]	+	[98, 99, 147, 219]	±	[98, 99]
CD49d	++/+	[73, 75, 219]	+	[98, 219]	±	[98]
CD73	+++	[85, 147, 220, 221]	+++	[147]		
CD90	+++	[85, 98, 150, 219–222]	+++	[98, 99, 147, 219]	+++	[98, 99]
CD105	++	[75, 85, 98, 150, 219–222]	++	[98, 99, 219]	++	[98, 99]
CD106	+/±	[73, 98, 219, 220, 222]	±	[98, 219]	±	[98]
CD117	+++/±	[73, 221]	±	*		
CD133	±	[73, 147, 172, 220]	±	[147, 172]	±	[172]
CD146	+	[85, 98, 222]	±	[98]	±	[98]
CD166	++/+	[75, 85, 98, 219, 221, 222]	+	[98, 219]	+	[98]
MHC I	+++/+	[73, 75, 98, 147, 172, 220]	+++/±	[98, 99, 147, 172, 219]	+++/+/±	[98, 99, 172]
MHC II	+/±	[73, 75, 98, 147, 220], *	±	[98, 99, 147], *	±	[98, 99]
STRO-1	+	[219, 222]	+	[219]		

FBS = fetal bovine serum, HS = human serum, SF = serum-free, XF = xeno-free

+++ = strong expression >90%, ++ = positive expression <90% >50%, + = moderate expression <50% >2%, ± = low or no expression ≤2%

* = Lindroos et al, unpublished results

B. Lindroos, R. Suurinen, S. Miettinen. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev and Rep*, 7:269-291. 2011.

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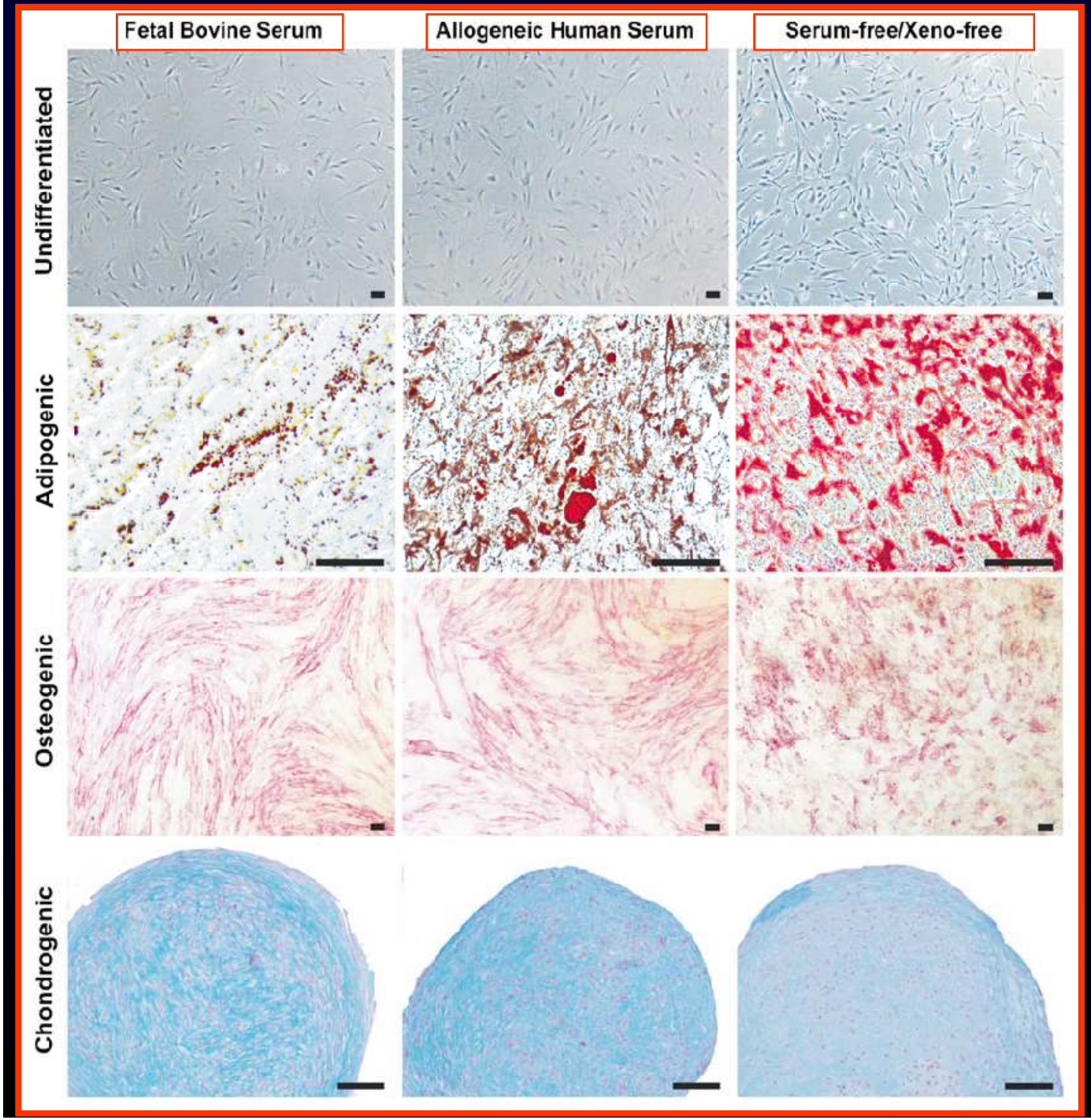

Culture-related AT-MSC variability

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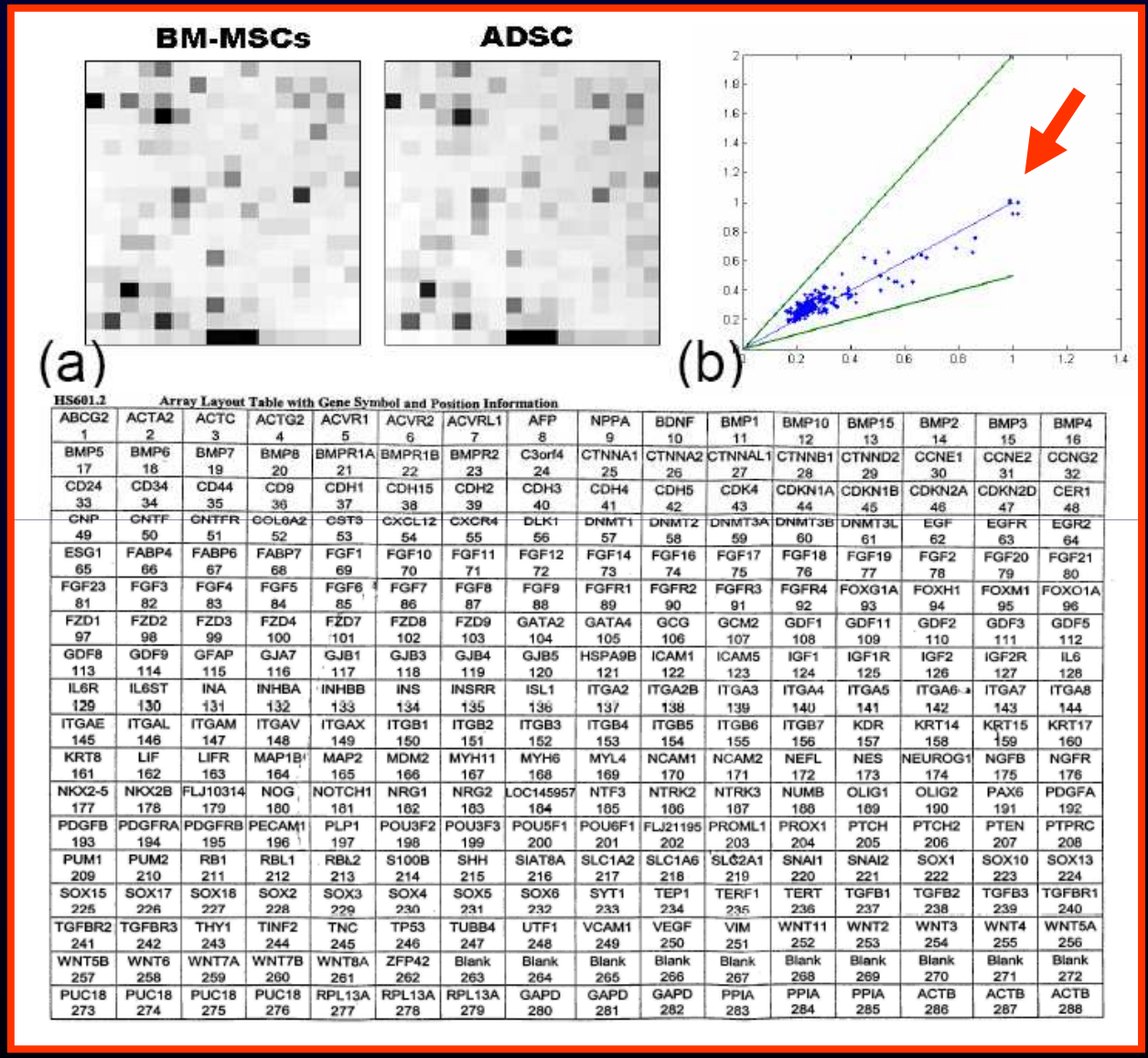
Adipose tissue-derived MSC (AT-MSC)



HS601.2 Array Layout Table with Gene Symbol and Position Information

ABCG2 1	ACTA2 2	ACTC 3	ACTG2 4	ACVR1 5	ACVR2 6	ACVRL1 7	AFP 8	NPPA 9	BDNF 10	BMP1 11	BMP10 12	BMP15 13	BMP2 14	BMP3 15	BMP4 16
BMP5 17	BMP6 18	BMP7 19	BMP8 20	BMPR1A 21	BMPR1B 22	BMPR2 23	C3orf4 24	CTNNA1 25	CTNNA2 26	CTNNAL1 27	CTNNB1 28	CTNND2 29	CCNE1 30	CCNE2 31	CCNG2 32
CD24 33	CD34 34	CD44 35	CD9 36	CDH1 37	CDH15 38	CDH2 39	CDH3 40	CDH4 41	CDH5 42	CDK4 43	CDKN1A 44	CDKN1B 45	CDKN2A 46	CDKN2D 47	CER1 48
CNP 49	CNTF 50	CNTFR 51	COL6A2 52	CST3 53	CXCL12 54	CXCR4 55	DLK1 56	DNMT1 57	DNMT2 58	DNMT3A 59	DNMT3B 60	DNMT3L 61	EGF 62	EGFR 63	EGR2 64
ESG1 65	FABP4 66	FABP6 67	FABP7 68	FGF1 69	FGF10 70	FGF11 71	FGF12 72	FGF14 73	FGF16 74	FGF17 75	FGF18 76	FGF19 77	FGF2 78	FGF20 79	FGF21 80
FGF23 81	FGF3 82	FGF4 83	FGF5 84	FGF6 85	FGF7 86	FGF8 87	FGF9 88	FGFR1 89	FGFR2 90	FGFR3 91	FGFR4 92	FOXG1A 93	FOXH1 94	FOXM1 95	FOXO1A 96
FZD1 97	FZD2 98	FZD3 99	FZD4 100	FZD7 101	FZD8 102	FZD9 103	GATA2 104	GATA4 105	GCG 106	GCM2 107	GDF1 108	GDF11 109	GDF2 110	GDF3 111	GDF5 112
GDF8 113	GDF9 114	GFAP 115	GJA7 116	GJB1 117	GJB3 118	GJB4 119	GJB5 120	HSPA9B 121	ICAM1 122	ICAM5 123	IGF1 124	IGF1R 125	IGF2 126	IGF2R 127	IL6 128
IL6R 129	IL6ST 130	INA 131	INHBA 132	INHBB 133	INS 134	INSRR 135	ISL1 136	ITGA2 137	ITGA2B 138	ITGA3 139	ITGA4 140	ITGA5 141	ITGA6 142	ITGA7 143	ITGA8 144
ITGAE 145	ITGAL 146	ITGAM 147	ITGAV 148	ITGAX 149	ITGB1 150	ITGB2 151	ITGB3 152	ITGB4 153	ITGB5 154	ITGB6 155	ITGB7 156	KDR 157	KRT14 158	KRT15 159	KRT17 160
KRT8 161	LIF 162	LIFR 163	MAP1B 164	MAP2 165	MDM2 166	MYH11 167	MYH6 168	MYL4 169	NCAM1 170	NCAM2 171	NEFL 172	NES 173	NEUROG1 174	NGFB 175	NGFR 176
NKX2-5 177	NKX2B 178	FLJ10314 179	NOG 180	NOTCH1 181	NRG1 182	NRG2 183	LOC145957 184	NTF3 185	NTRK2 186	NTRK3 187	NUMB 188	OLIG1 189	OLIG2 190	PAX6 191	PDGFA 192
PDGFB 193	PDGFRA 194	PDGFRB 195	PECAM1 196	PLP1 197	POU3F2 198	POU3F3 199	POU5F1 200	POU6F1 201	FLJ21195 202	PROML1 203	PROX1 204	PTCH 205	PTCH2 206	PTEN 207	PTPRC 208
PUM1 209	PUM2 210	RB1 211	RBL1 212	RBL2 213	S100B 214	SHH 215	SIAT8A 216	SLC1A2 217	SLC1A6 218	SLC2A1 219	SNAI1 220	SNAI2 221	SOX1 222	SOX10 223	SOX13 224
SOX15 225	SOX17 226	SOX18 227	SOX2 228	SOX3 229	SOX4 230	SOX5 231	SOX6 232	SYT1 233	TEP1 234	TERF1 235	TERT 236	TGFB1 237	TGFB2 238	TGFB3 239	TGFBR1 240
TGFBR2 241	TGFBR3 242	THY1 243	TINF2 244	TNC 245	TP53 246	TUBB4 247	UTF1 248	VCAM1 249	VEGF 250	VIM 251	WNT11 252	WNT2 253	WNT3 254	WNT4 255	WNT5A 256
WNT5B 257	WNT6 258	WNT7A 259	WNT7B 260	WNT8A 261	ZFP42 262	Blank 263	Blank 264	Blank 265	Blank 266	Blank 267	Blank 268	Blank 269	Blank 270	Blank 271	Blank 272
PUC18 273	PUC18 274	PUC18 275	PUC18 276	RPL13A 277	RPL13A 278	RPL13A 279	GAPD 280	GAPD 281	GAPD 282	PPIA 283	PPIA 284	PPIA 285	ACTB 286	ACTB 287	ACTB 288

D. Peroni, I. Scambi, A. Pasini, V. Lisi, F. Bifari, M. Krampera, G. Rigotti, A. Sbarbati, M. Galiè. Stem molecular signature of adipose-derived stromal cells. *Exp Cell Research 2008; 314: 603-615.*

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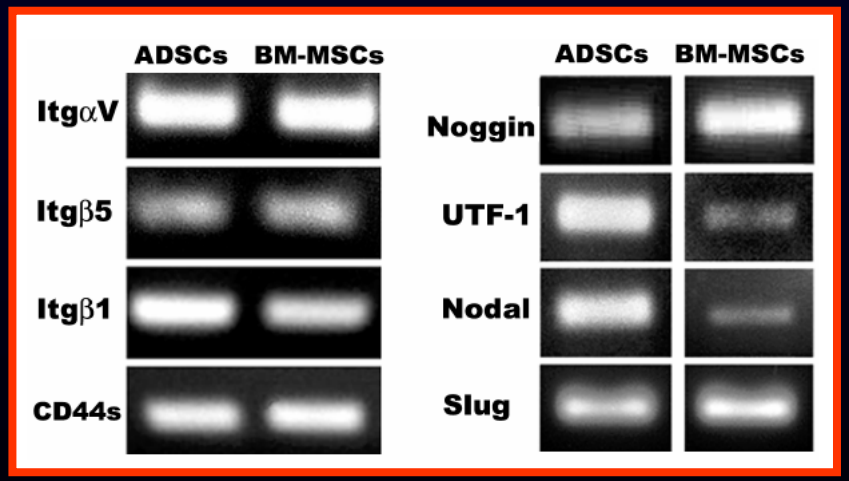
Adipose tissue-derived MSC (AT-MSC)

- Integrins and adhesion molecules (α5, αV, β1, β5, CD44 isoforms)
- Cytokines/chemokines (CxCL12/SDF-1, IL-6, CCL2/MCP-1, CCL7/MCP-3, CxCL1/Gro-α, CxCL12/Gro-β)
- Cyclin-dependent kinase inhibitors (CDK 1A/$p21^{cip1}$, 1B/$p27^{Kip1}$, 2A/p16, 2D/p19)
- Growth Factors (FGF-2/16/5/6, VEGF, PDGF-A)
- Bone Morphogenetic Protein Receptors (BMPR-2)
- Embryonic factors (Slug/Snail2 - migration neural crest cells, E-M transition in cancers)
- Metalloproteinases (MMP-12, -14, -2, -23, -3)

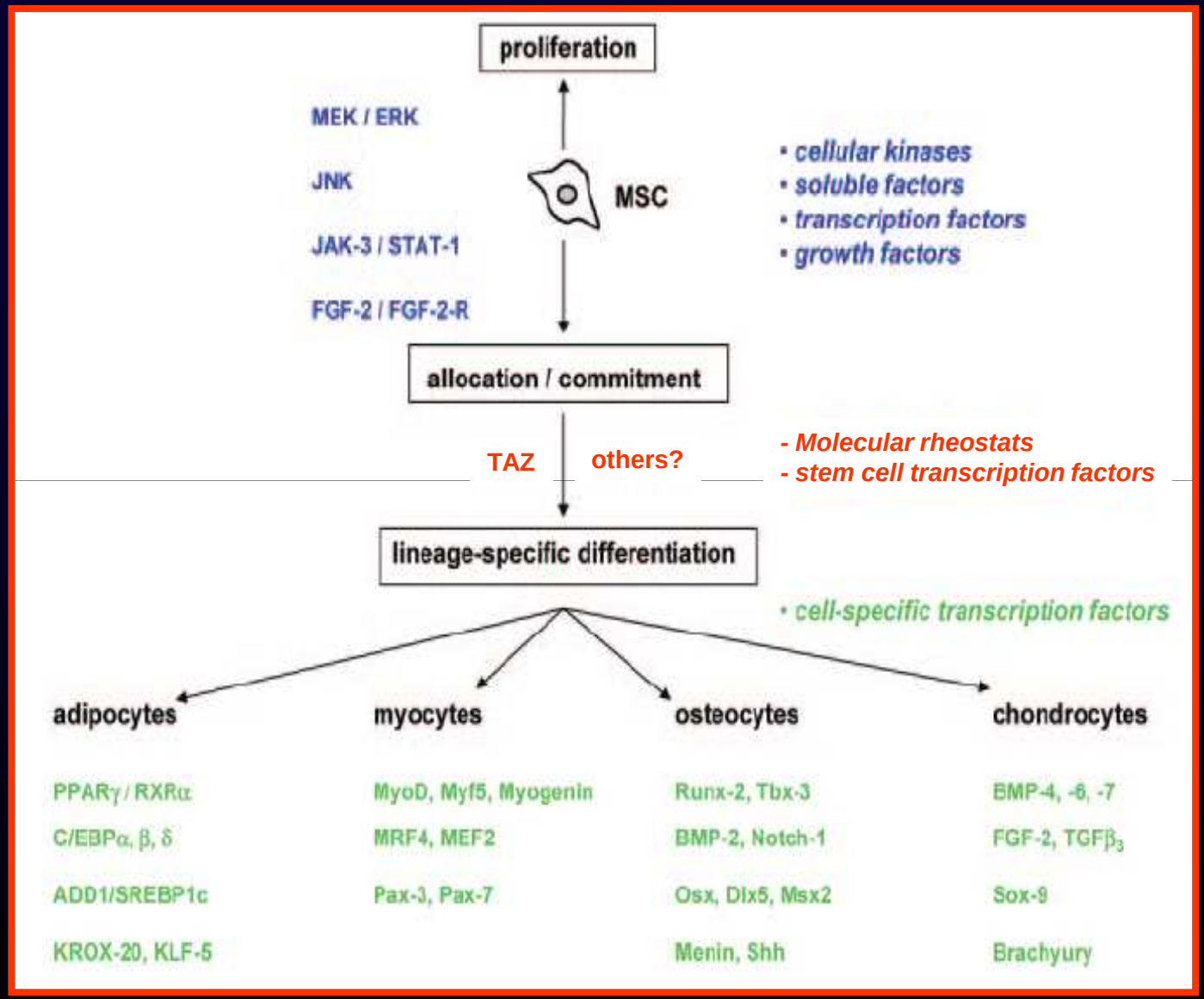


(RT-PCR)

D. Peroni, I. Scambi, A. Pasini, V. Lisi, F. Bifari, M. Krampera, G. Rigotti, A. Sbarbati, M. Galiè. Stem molecular signature of adipose-derived stromal cells. *Exp Cell Research 2008; 314: 603-615.*

Krampera

Adipose tissue-derived MSC (AT-MSC)



A. Schaffler, C. Buchler. Concise Review: Adipose Tissue-Derived Stromal Cells – Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* 2007; 25:818-827.

Krampera

Adipose tissue-derived MSC (AT-MSC) - differentiation assays -

Table 3. Experimentally used factors triggering the differentiation of adipose tissue-derived stromal cells

Type of differentiation	Differentiation factors
Adipogenic	Insulin, IBMX, dexamethasone, rosiglitazone, indomethacin
Chondrogenic	BMP-6, BMP-7, FGF-2, TGF-β_1, TGF-β_2, TGF-β_3, dexamethasone, IGF-1
Osteogenic	1,25$(OH)_2D_3$, β-glycerophosphate, ascorbic acid, BMP-2, dexamethasone, valproic acid
Myogenic differentiation	Specific microenvironment?
Cardiomyogenic differentiation	IL-3, IL-6, SCF
Vascular/endothelial	Specific microenvironment?
Neurogenic	Valproic acid, insulin, hydroxyanisole, hydrocortisone, EGF, FGF
Pancreatic/endocrine	Activin-A, exendin-4, pentagastrin, HGF, nicotinamide, high glucose concentration
Hepatic	HGF, OSM, DMSO
Hematopoietic	Specific microenvironment?

Abbreviations: 1,25$(OH)_2D_3$, 1,25-dihydroxy-cholecalciferol; BMP, bone morphogenetic protein; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IBMX, 3-isobutyl-1-methylxanthine; IGF, insulin-like growth factor; IL, interleukin; OSM, oncostatin M; SCF, stem cell factor; TGF, transforming growth factor.

A. Schaffler, C. Buchler. Concise Review: Adipose Tissue-Derived Stromal Cells – Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* 2007; 25:818-827.

Adipose tissue-derived MSC (AT-MSC)

Type of differentiation	Clinical implications
Adipogenic	Breast soft tissue reconstruction after tumor surgery for breast cancer, breast asymmetry, and soft tissue and subdermal defects after trauma, surgery, or burn injury
Chondrogenic	Cartilage repair in joint and disc defects, plastic reconstruction of ear and nose defects
Osteogenic	Skeletal regeneration of inherited and tumor- or trauma-induced bone defects
Myogenic	Tissue reconstruction after trauma and surgery, dystrophic muscle disorders
Cardiomyogenic	Heart muscle regeneration, functional improvement after myocardial infarction, heart failure
Vascular/endothelial	Neovascularization, ischemic diseases
Neurogenic	Brain injury, stroke, peripheral nerve injury
Pancreatic/endocrine	Insulin-secreting cells, type 1 diabetes mellitus
Hepatic	Chronic liver failure, hepatic regeneration, hepatocyte transplantation
Hematopoietic	GVHD, bone marrow support

A. Schaffler, C. Buchler. Concise Review: Adipose Tissue-Derived Stromal Cells – Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* 2007; 25:818-827.

Immune regulation

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Neural differentiation of MSCs of various origin by neurogenic medium

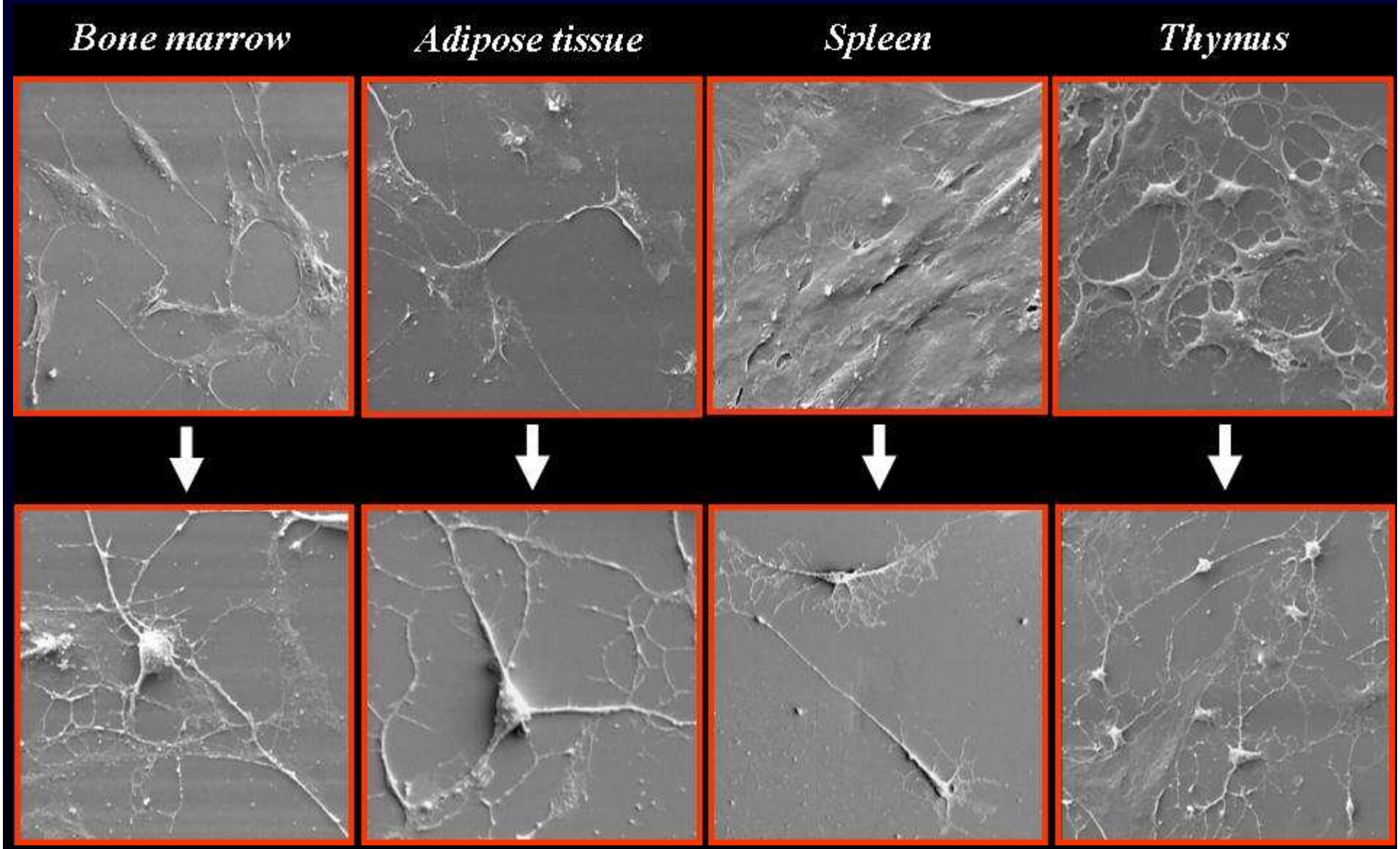
(bone marrow, adipose tissue, spleen, thymus)

Woodbury's modified protocol:

- pre-induction medium: DMEM + FBS 10% + 5 ng/ml bFGF for 24 h
- wash with PBS
- induction medium: DMEM + N2 supplement, butylated hydroxyanisole, KCl, valproic acid and forskolin for 2 to 16 h
- cell fixation for immunocytochemistry or transfer to basal medium to assess their phenotype and differentiation potential

M. Krampera, S. Marconi, A. Pasini, M. Galiè,G., et al. Induction of neural-like differentiation in human MSCs derived from bone marrow, fat, spleen and thymus. *Bone* 2007; 40: 382-390.

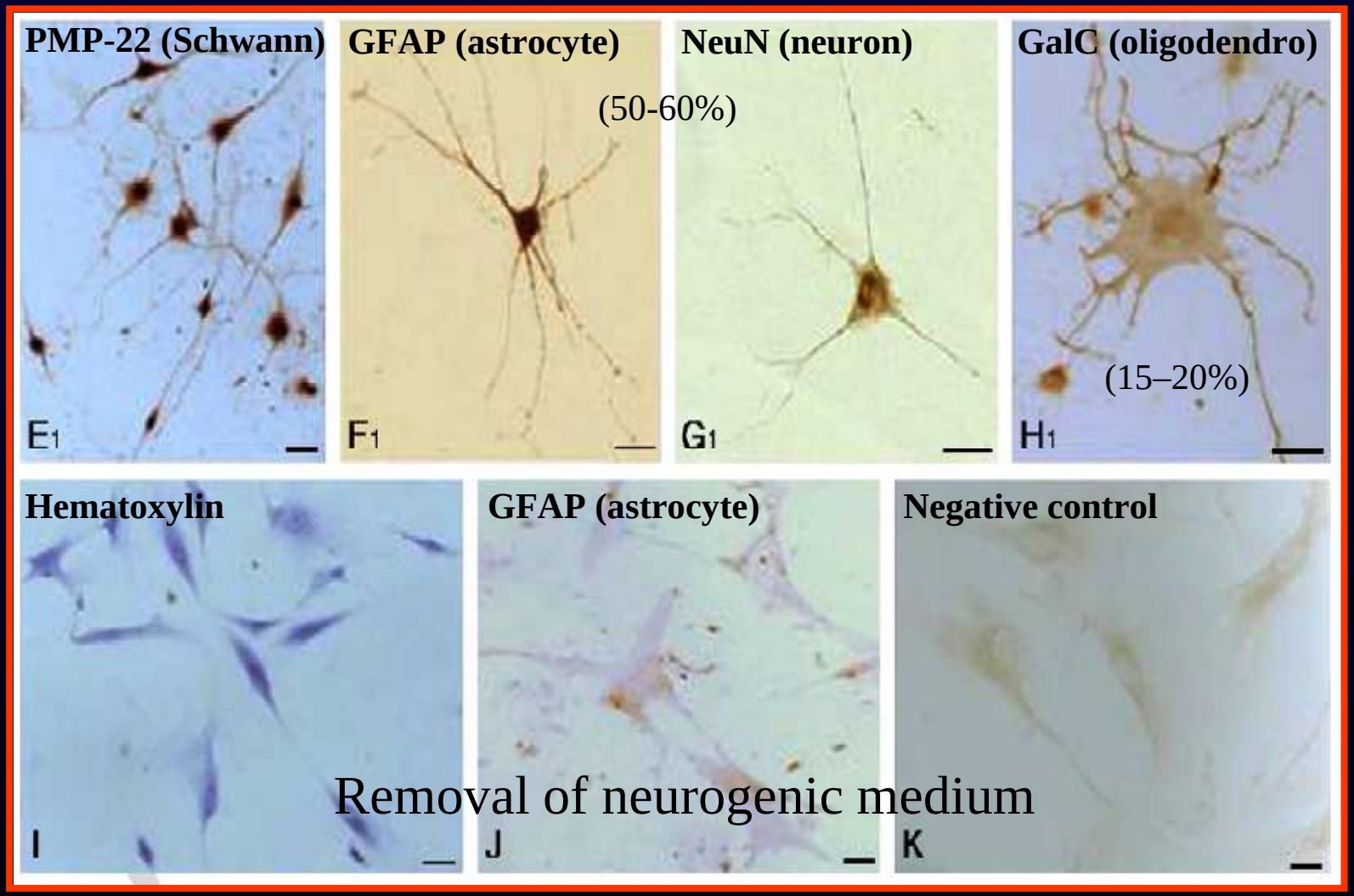
Neural differentiation of MSCs of various origin by neurogenic medium



M. Krampera, S. Marconi, A. Pasini, M. Galiè,G., et al. Induction of neural-like differentiation in human MSCs derived from bone marrow, fat, spleen and thymus. *Bone* 2007; 40: 382-390.

Krampera

Neural differentiation of MSCs of various origin by neurogenic medium



M. Krampera, S. Marconi, A. Pasini, M. Galiè,G., et al. Induction of neural-like differentiation in human MSCs derived from bone marrow, fat, spleen and thymus. *Bone* 2007; 40: 382-390.

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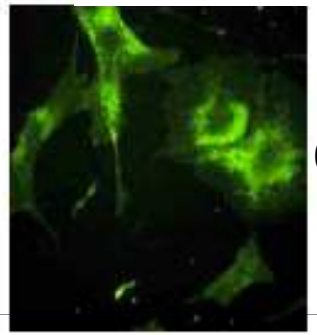
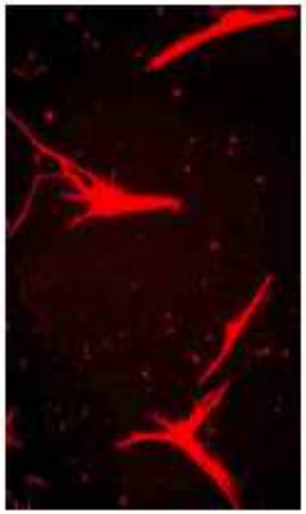
Neural differentiation of MSCs of various origin by co-culture with Schwann cells



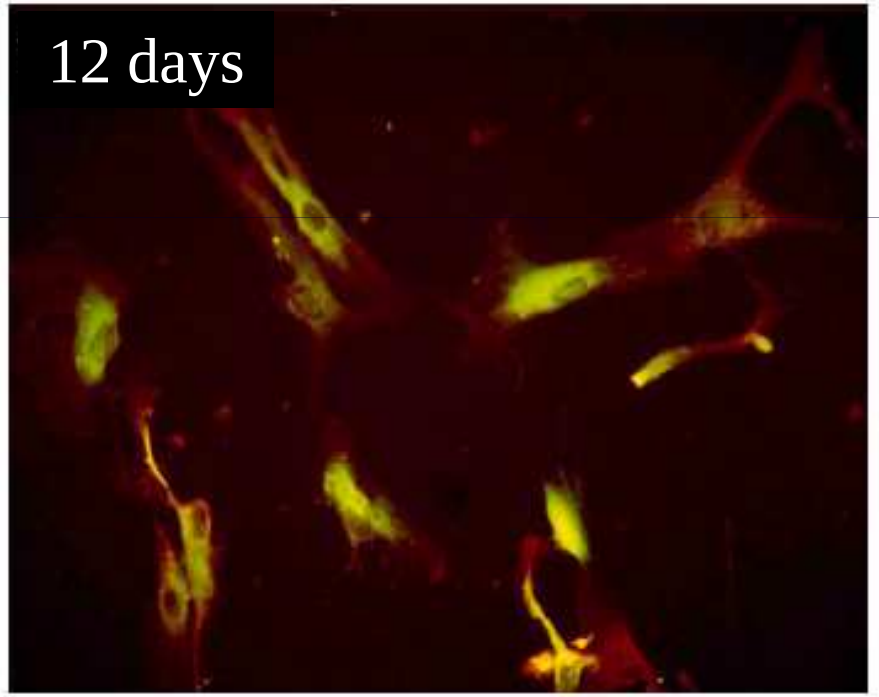
Human MSCs
(**PKH-67-labelled**)



Co-culture
(4, 7, 12 days)

Human Schwann cells
(**PMP-22+** or **S100+**)

(25-30% delle AT-MSC,
> se pre-induzione con medium neurogenico)



M. Krampera, S. Marconi, A. Pasini, M. Galiè,G., et al. Induction of neural-like differentiation in human MSCs derived from bone marrow, fat, spleen and thymus. *Bone* 2007; 40: 382-390.

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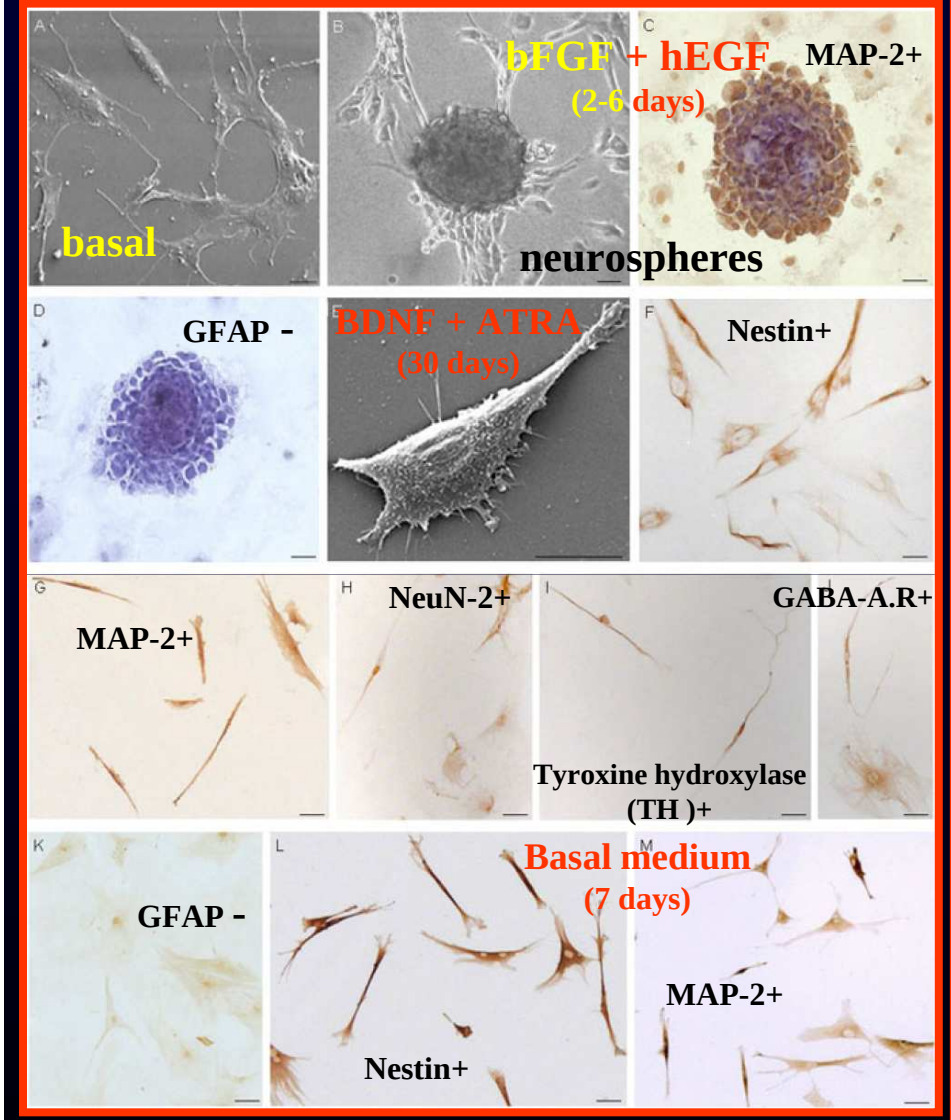
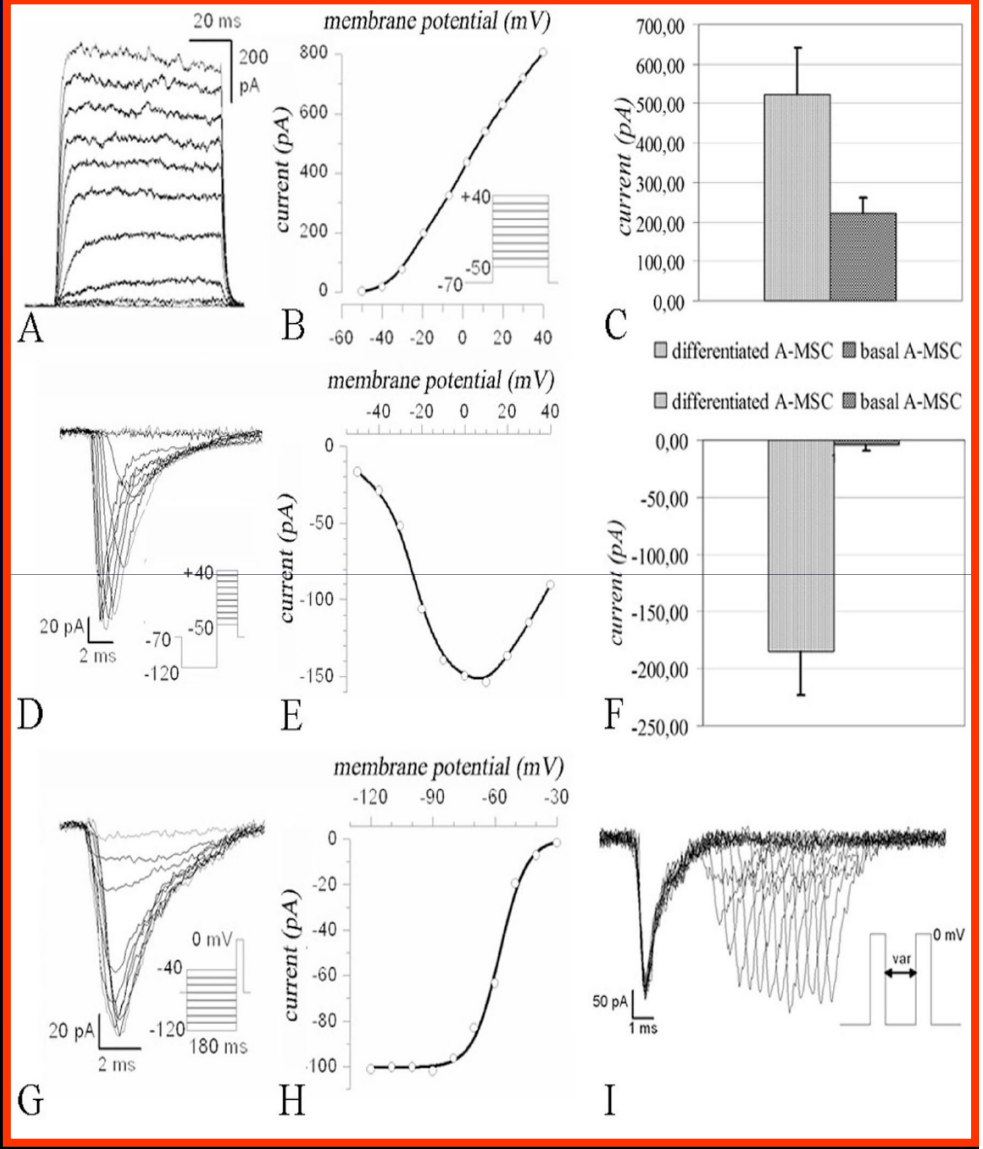
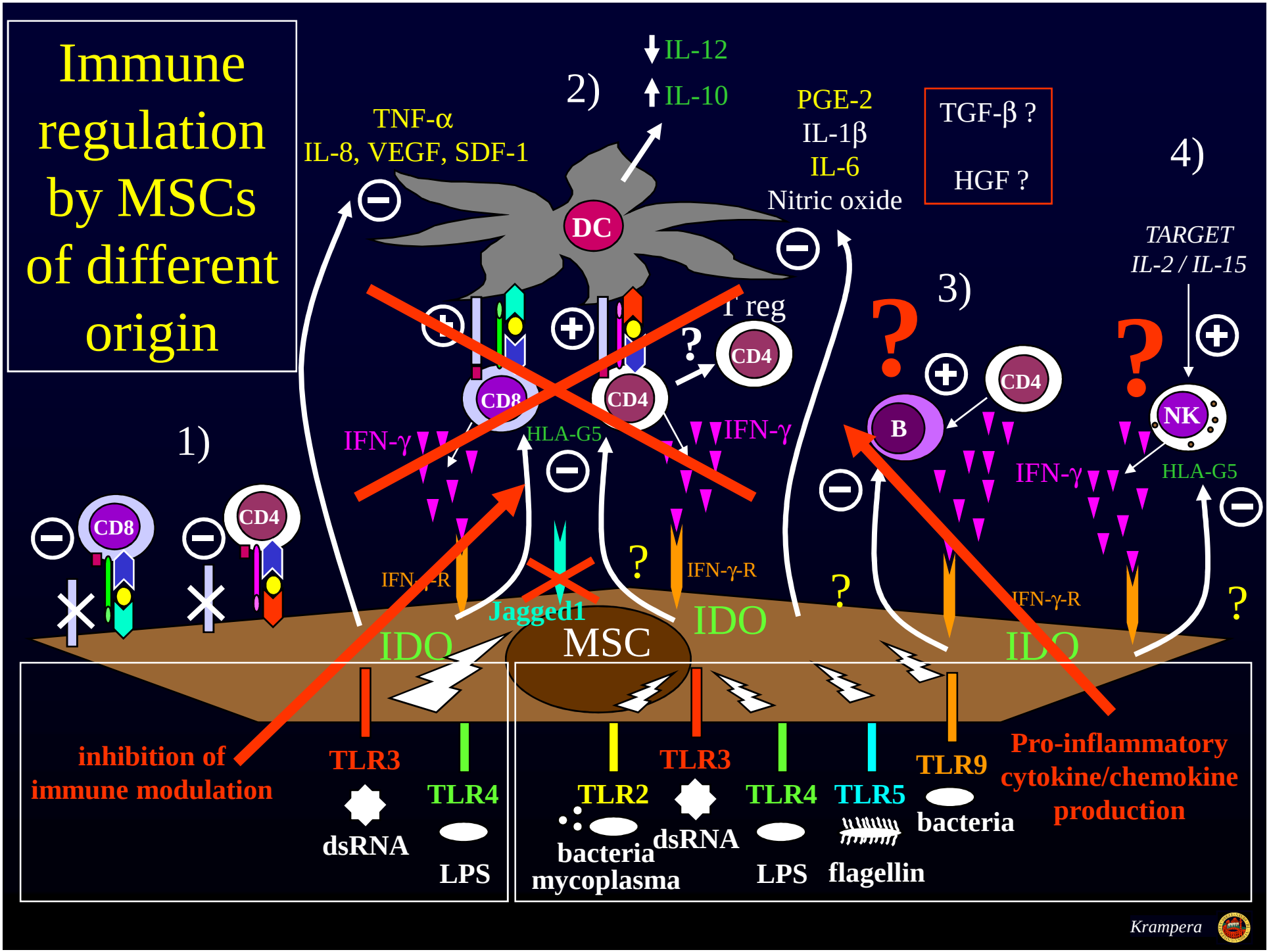
Neuronal differentiation of AT-MSC



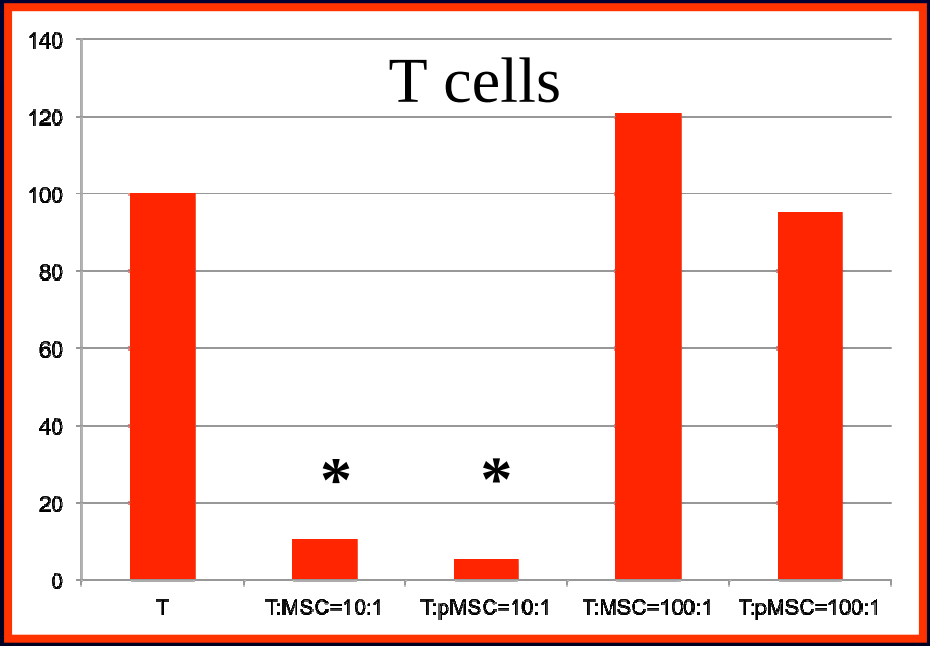


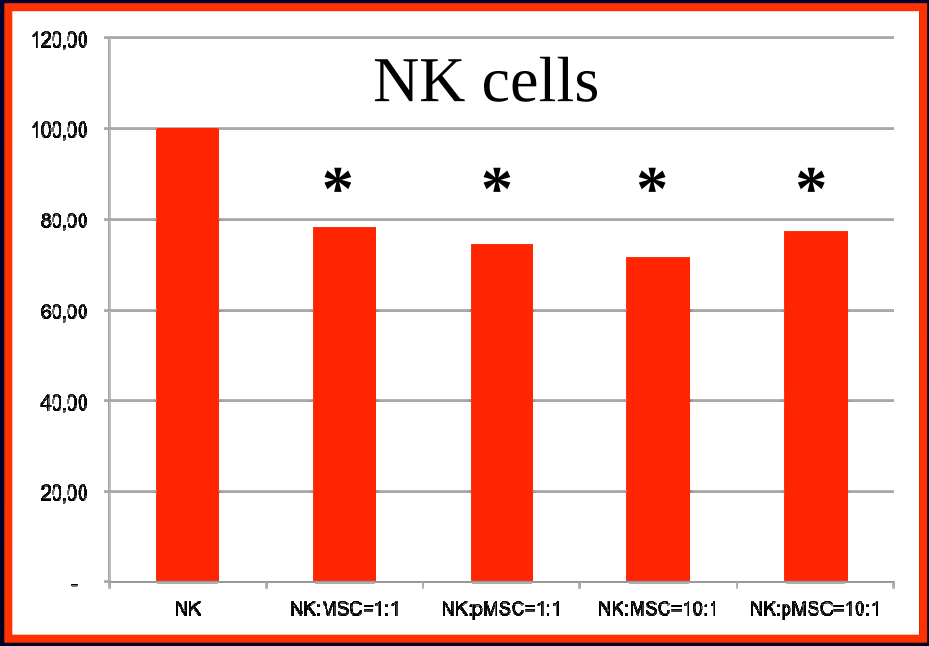
E. Anghileri, , S. Marconi, A. Pignatelli, P. Cifelli, M. Galié, A. Sbarbati, M. Krampera, O. Belluzzi, B. Bonetti.
Neuronal differentiation potential of human mesenchymal stem cells. *Stem Cells and Development 2008;17:909-916.*

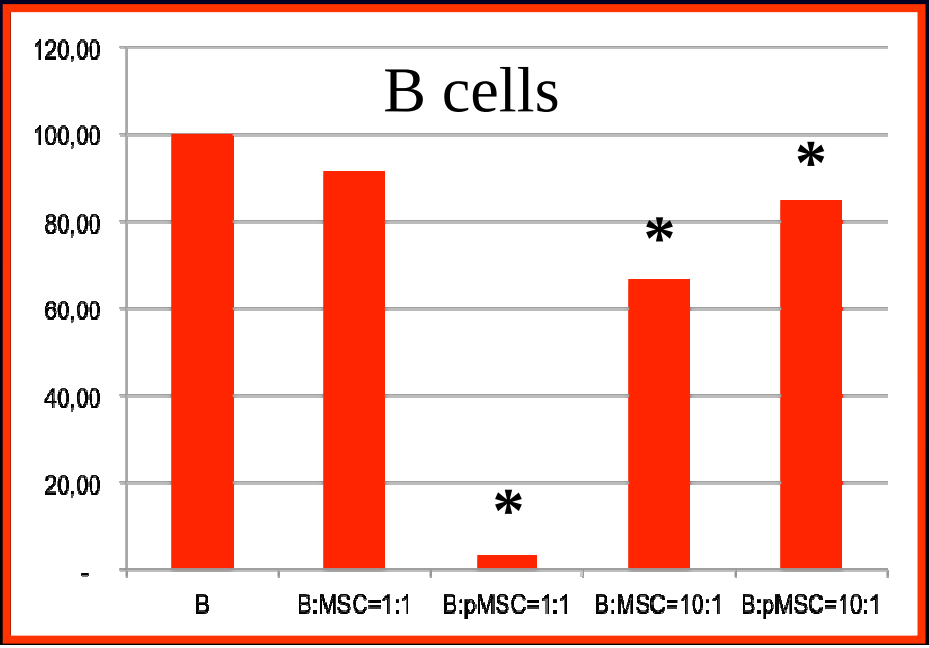
Krampera


Immune regulation by MSCs of different origin
1)
2)
3)
4)
IL-12
IL-10
TNF-α
IL-8, VEGF, SDF-1
PGE-2
IL-1β
IL-6
Nitric oxide
TGF-β ?
HGF ?
DC
T reg
CD4
CD8
B
NK
IFN-γ
HLA-G5
TARGET
IL-2 / IL-15
IFN-γ-R
Jagged1
IDO
MSC
inhibition of immune modulation
TLR3
dsRNA
TLR4
LPS
TLR2
bacteria mycoplasma
TLR5
flagellin
TLR9
bacteria
Pro-inflammatory cytokine/chemokine production
Krampera

AT-MSC inhibitory effect







MSC: unprimed

pMSC: MSC primed with IFN-γ and TNF-α

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Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

Lancet 2004 **363:** 1439–41

Katarina Le Blanc, Ida Rasmusson, Berit Sundberg, Cecilia Götherström, Moustapha Hassan, Mehmet Uzunel, Olle Ringdén

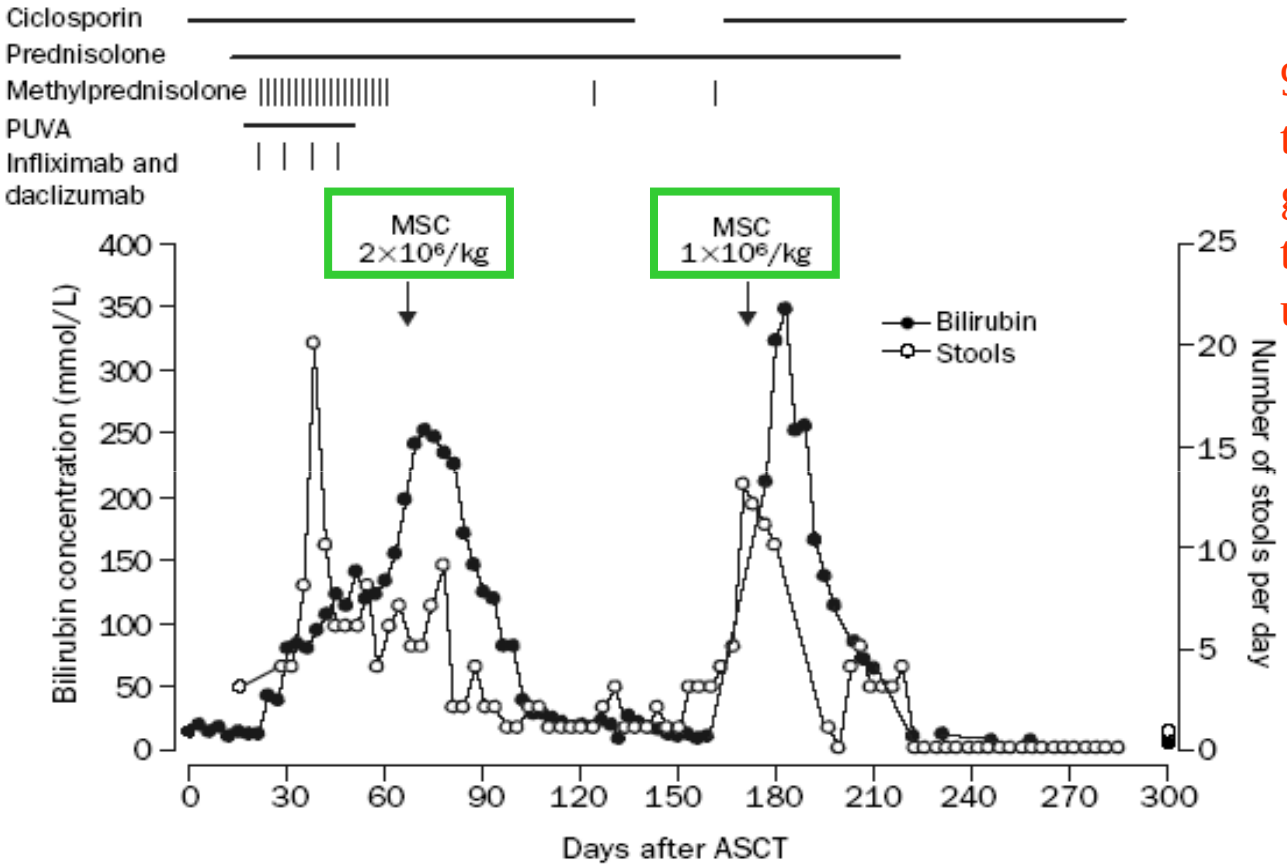


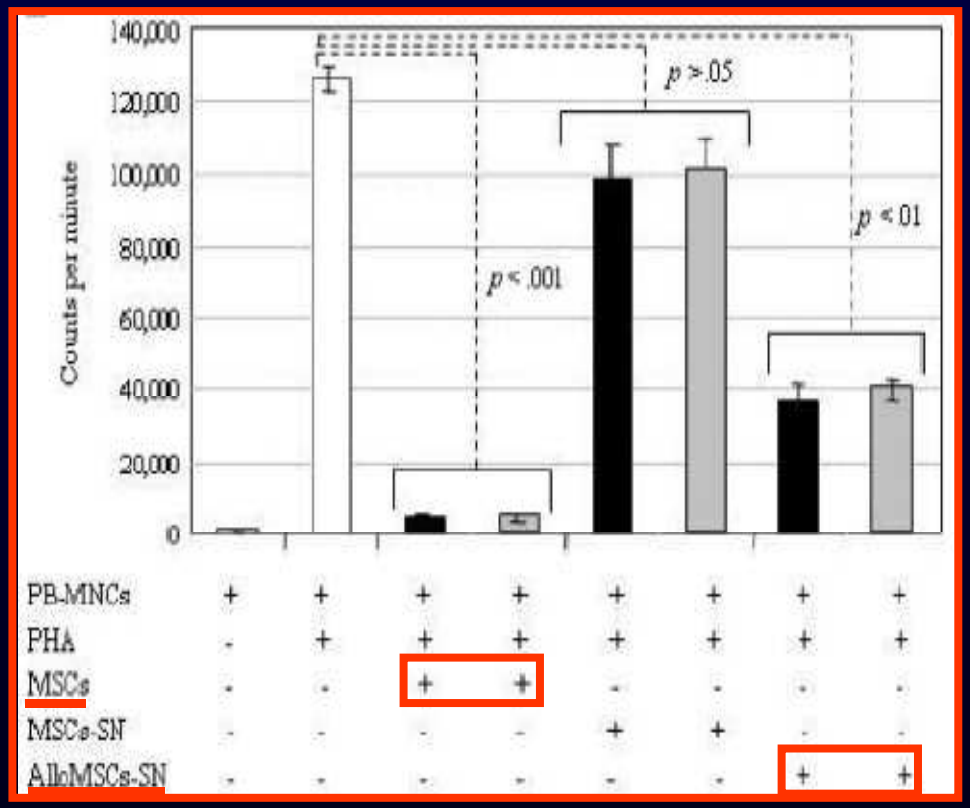
Figure 1: **Clinical course and immunosuppression of the patient**
↓=mesenchymal stem-cell transplantation. ASCT=allogeneic stem-cell transplantation. MSC=mesenchymal stem cells.

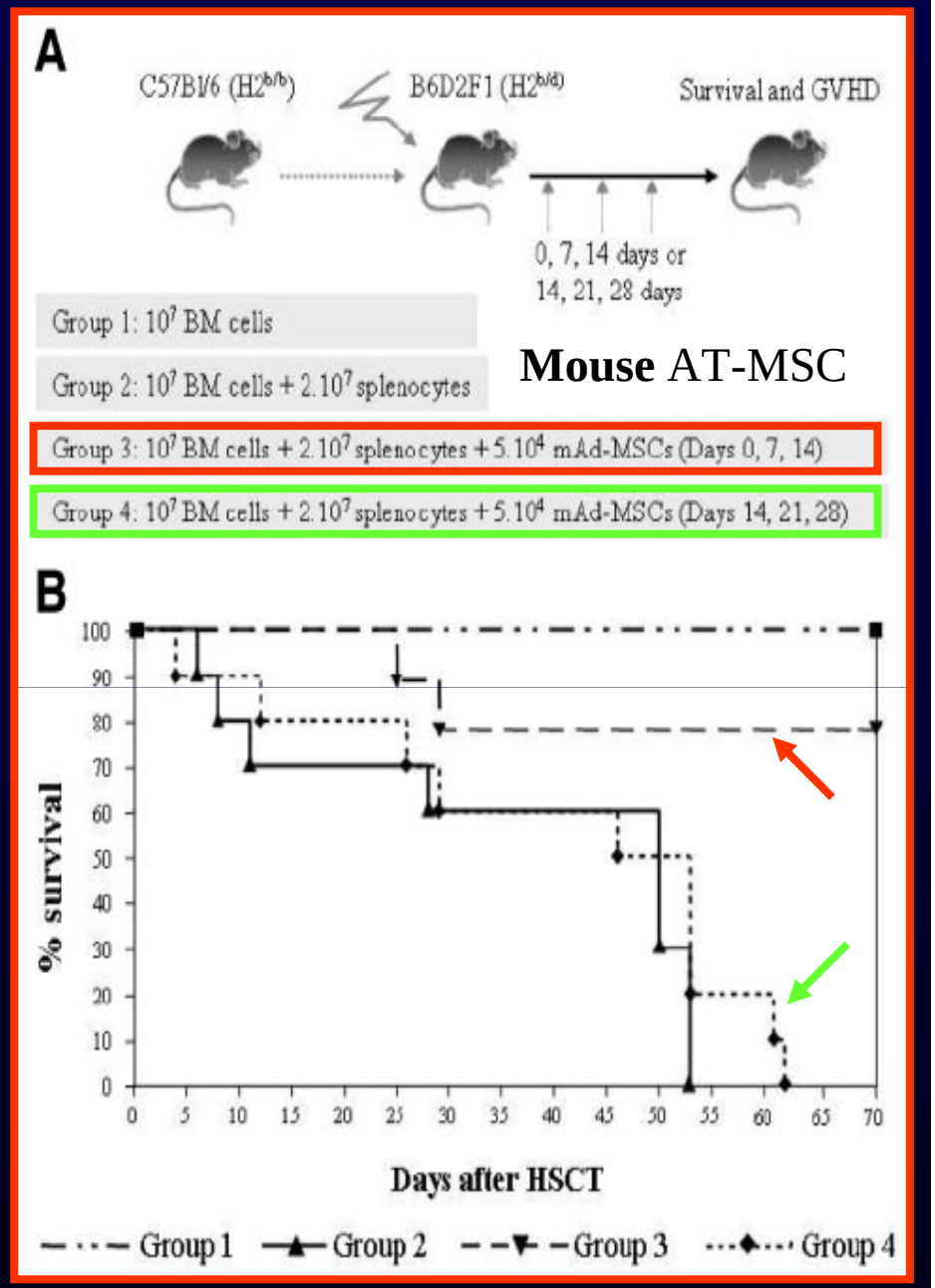
9-year patient with severe, treatment-resistant, grade IV acute GvHD of the gut and liver, following unrelated identical ASCT

Ringden O, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation 2006;81:1390-7.
Le Blanc K, et al. MSCs for treatment of steroid-resistant, severe, aGvHD: a phase II study. Lancet 2008;371:1579-86.

AT-MSC control GvHD in haploidentical hematopoietic grafts

Human AT-MSC





Mouse AT-MSC

Yanez, R, et al. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006;24:2582–2591

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Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy

BLOOD, 1 SEPTEMBER 2005 · VOLUME 106, NUMBER 5

Emanuela Zappia, Simona Casazza, Enrico Pedemonte, Federica Benvenuto, Ivan Bonanni, Ezio Gerdoni, Debora Giunti, Antonella Ceravolo, Francesco Cazzanti, Francesco Frassoni, Gianluigi Mancardi, and Antonio Uccelli

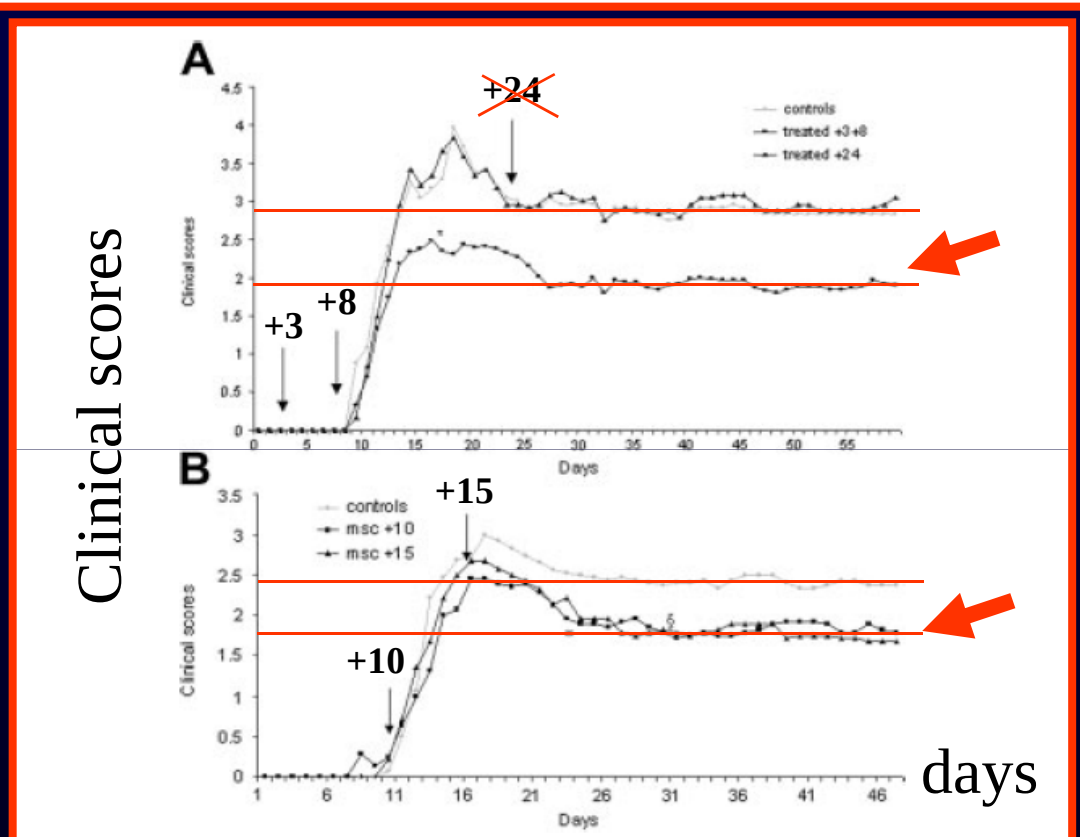
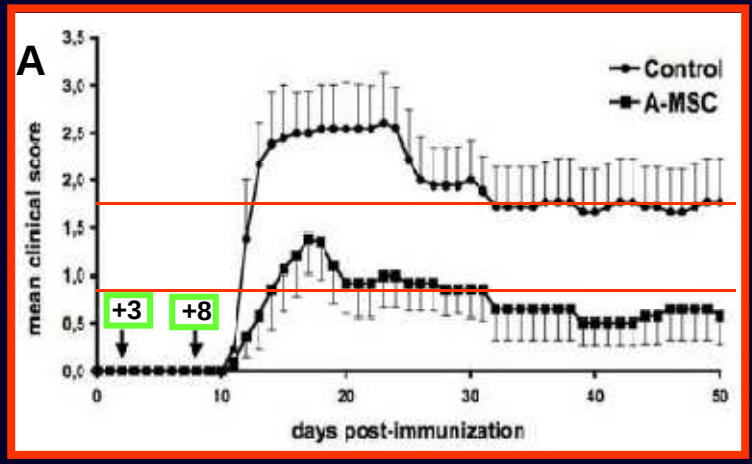


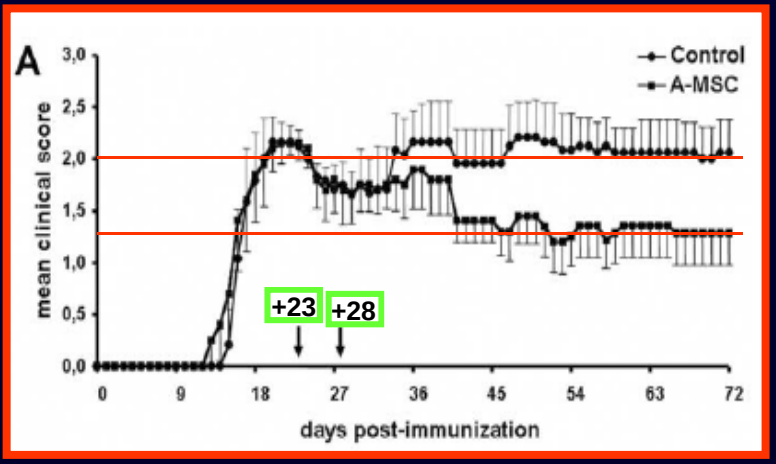
Figure 3. Intravenous injection of MSCs ameliorates EAE. MSCs injected at days 3 and 8 after immunization (■) halt disease severity compared with controls (△) ($P < .05$ from day 17 onward). No differences were observed between controls and mice treated at day 24 after immunization (▲) (A). Administration of MSCs at day 10 (■) or at day 15 (▲) after immunization, after disease onset, ameliorates EAE ($P < .05$ from day 22 [*] for mice treated at day 10 and from day 30 [§] for mice treated at day 15 compared with controls [△] by Mann-Whitney *U* test) (B). Arrows indicate days of MSC injection.

EAE prevention

EAE treatment

AT-MSC prevent and treat EAE

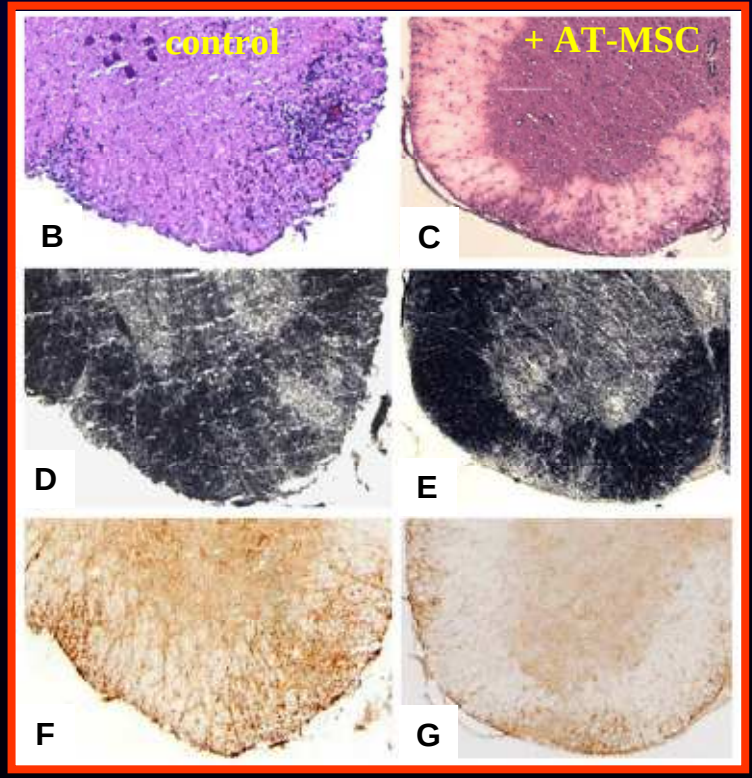


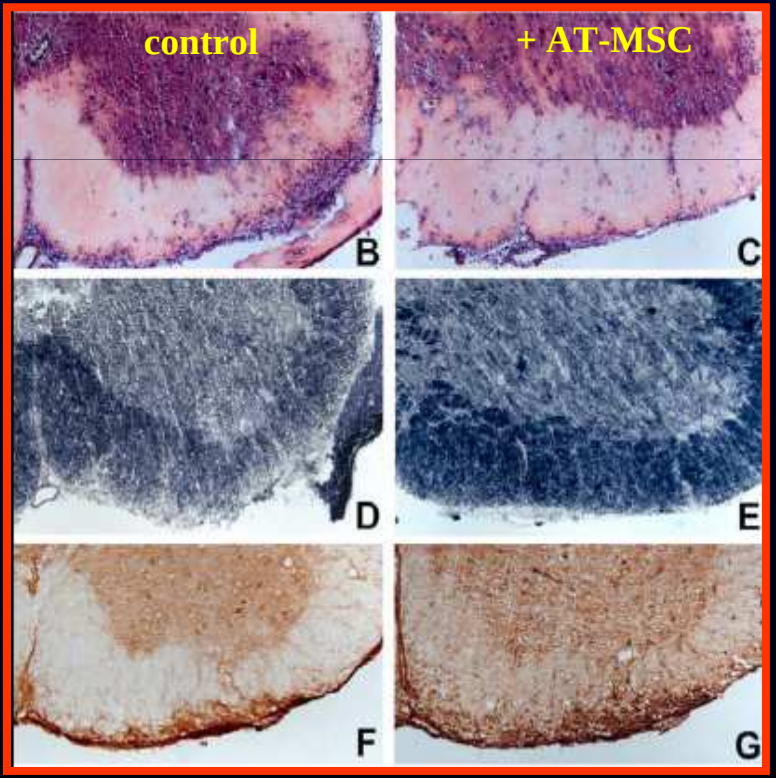


Hematoxylin-eosin

Spielmeyer

Anti-CD3





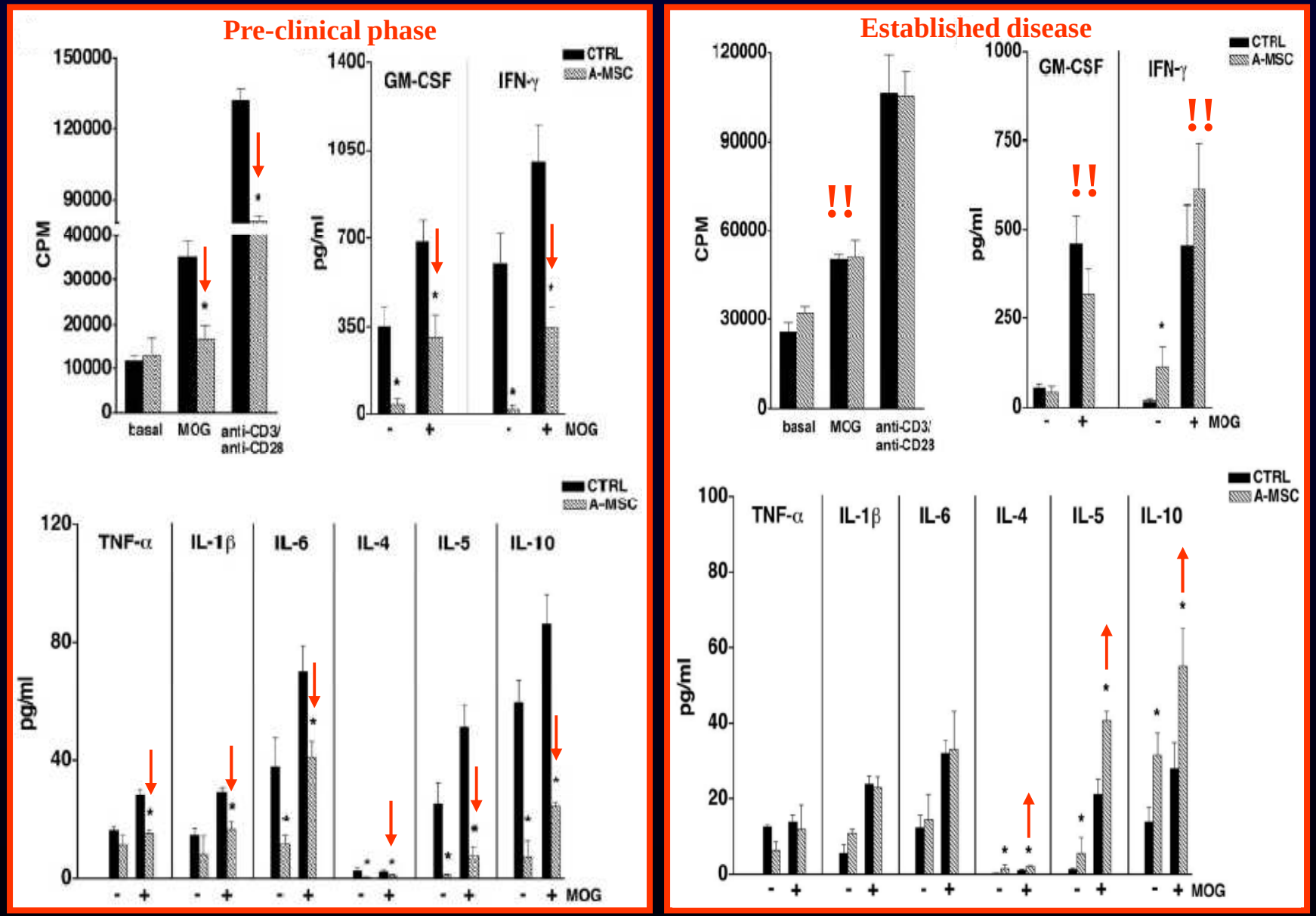
Hematoxylin-eosin

Spielmeyer

Anti-PDGFαR (oligodendro precursors)

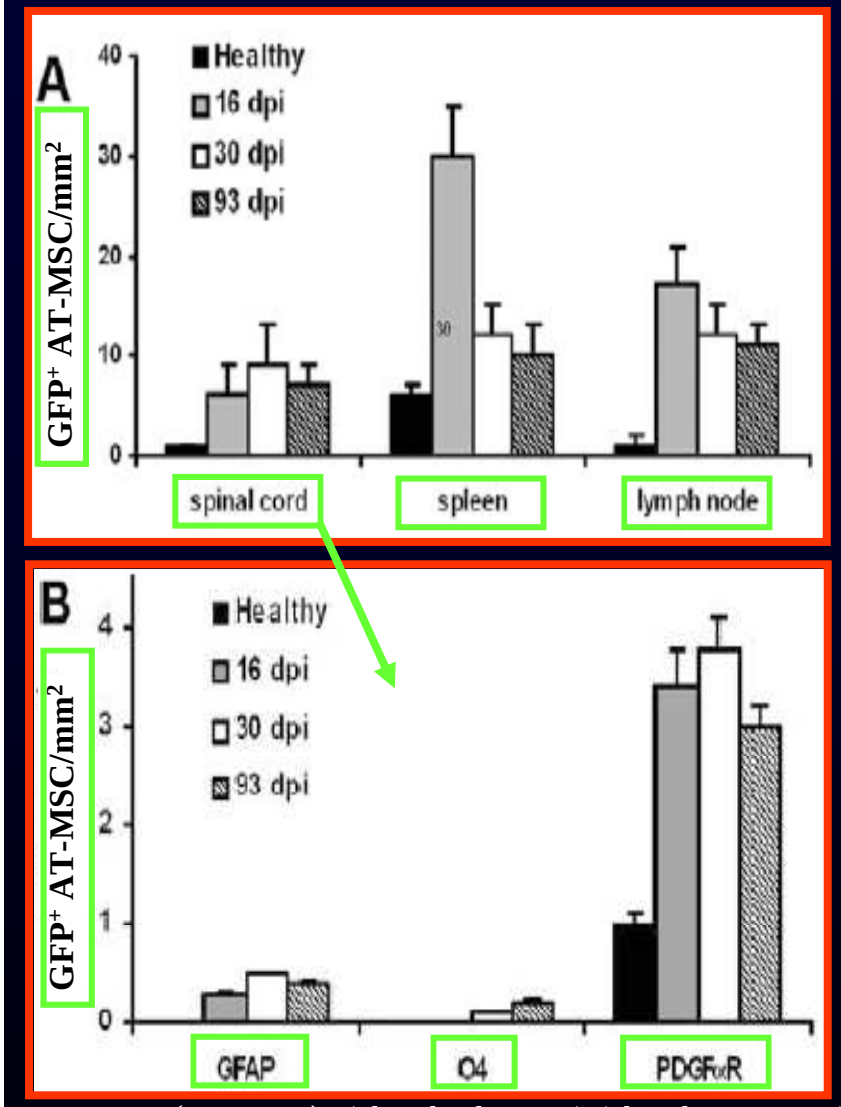
Constantin G, Marconi S, Rossi B, Angiari S, Gini B, Anghileri E, Bach SD, Martinello M, Bifari F, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. AT-MSC ameliorate chronic established EAE. *Stem Cells* 2009;27:2624-35 *Krampera*

AT-MSC in EAE: *ex-vivo* inhibitory effects



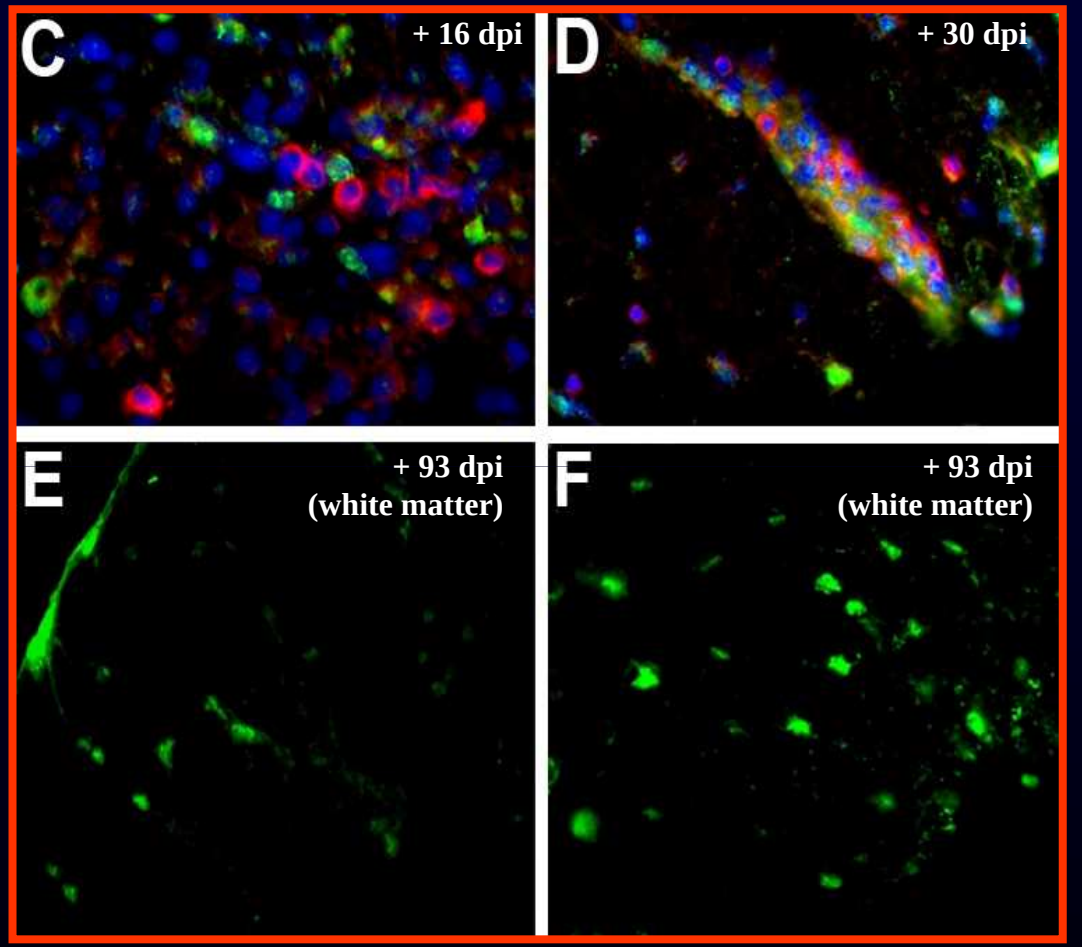
Constantin G, Marconi S, Rossi B, Angiari S, Gini B, Anghileri E, Bach SD, Martinello M, Bifari F, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. AT-MSC ameliorate chronic established EAE. *Stem Cells* 2009;27:2624-35 *Krampera*

AT-MSC in EAE: *in vivo* distribution of GFP$^+$ AT-MSC



(astrocytes) (oligodendrocytes) (oligod. precursors)

DAPI, CD3, GFP (perivascular cuffs of spinal cord in EAE mice)



Constantin G, Marconi S, Rossi B, Angiari S, Gini B, Anghileri E, Bach SD, Martinello M, Bifari F, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. AT-MSC ameliorate chronic established EAE. *Stem Cells* 2009;27:2624-35

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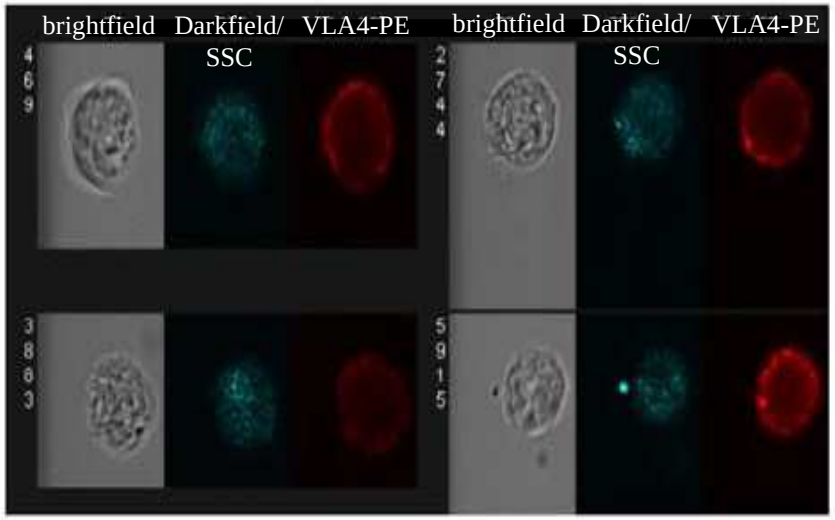
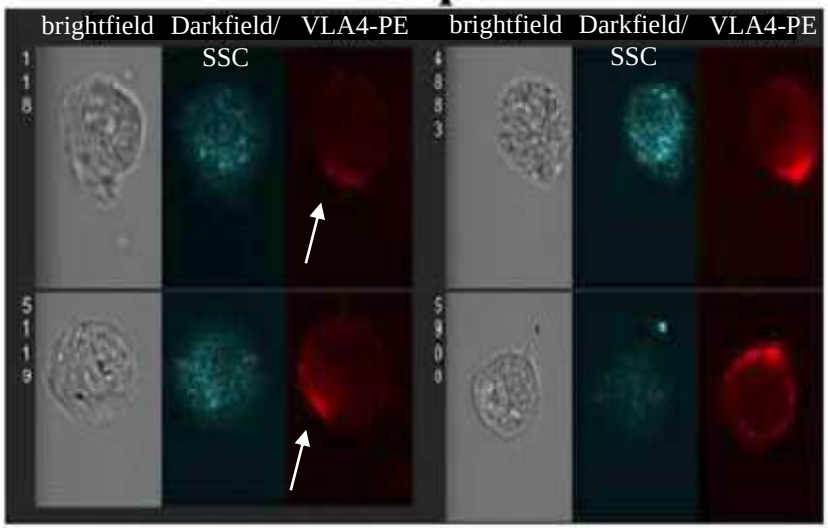
AT-MSC are VLA-4 ($\alpha4\beta1$)$^+$ and migrate into inflamed spinal cord

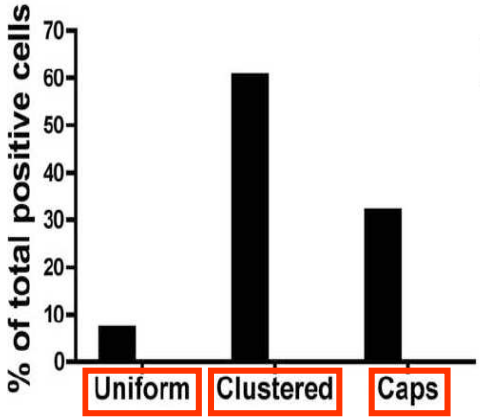
(through interaction with VCAM-1$^+$ endothelial cells)

ImageStream analysis **Clustered**

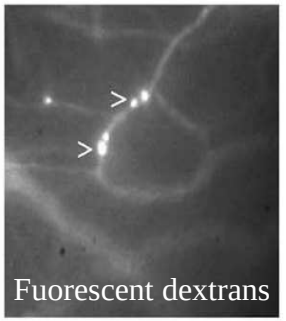


ImageStream analysis **Caps**

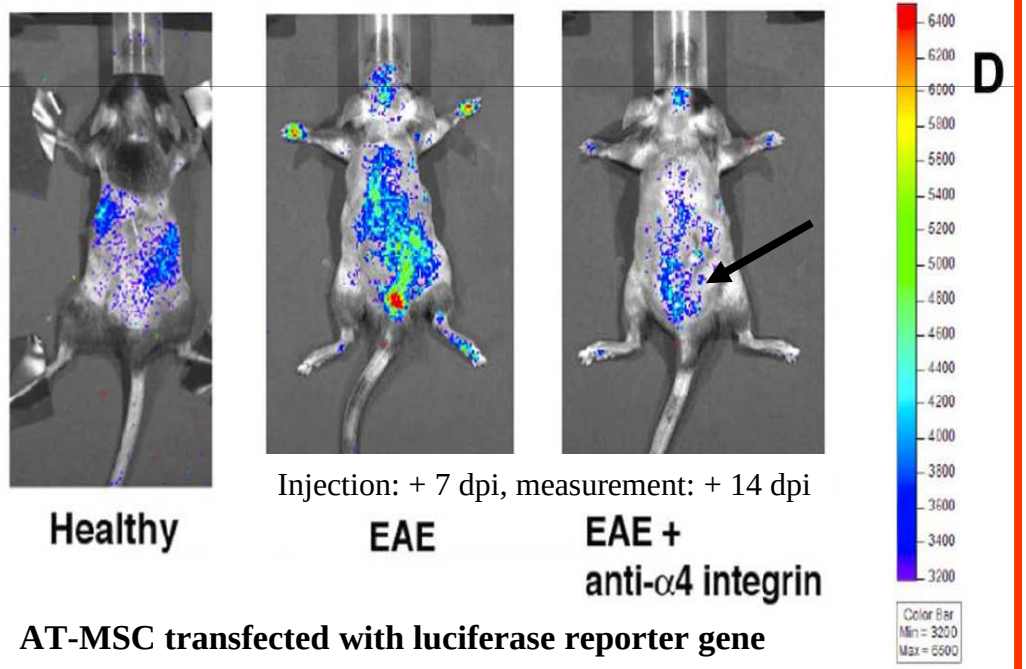




Intravital microscopy



IVIS 200



Injection: + 7 dpi, measurement: + 14 dpi

Healthy | EAE | EAE + anti-$\alpha4$ integrin

AT-MSC transfected with luciferase reporter gene

Constantin G, Marconi S, Rossi B, Angiari S, Gini B, Anghileri E, Bach SD, Martinello M, Bifari F, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. AT-MSC ameliorate chronic established EAE. *Stem Cells* 2009;27:2624-35 *Krampera*

In vivo use of AT-MSC

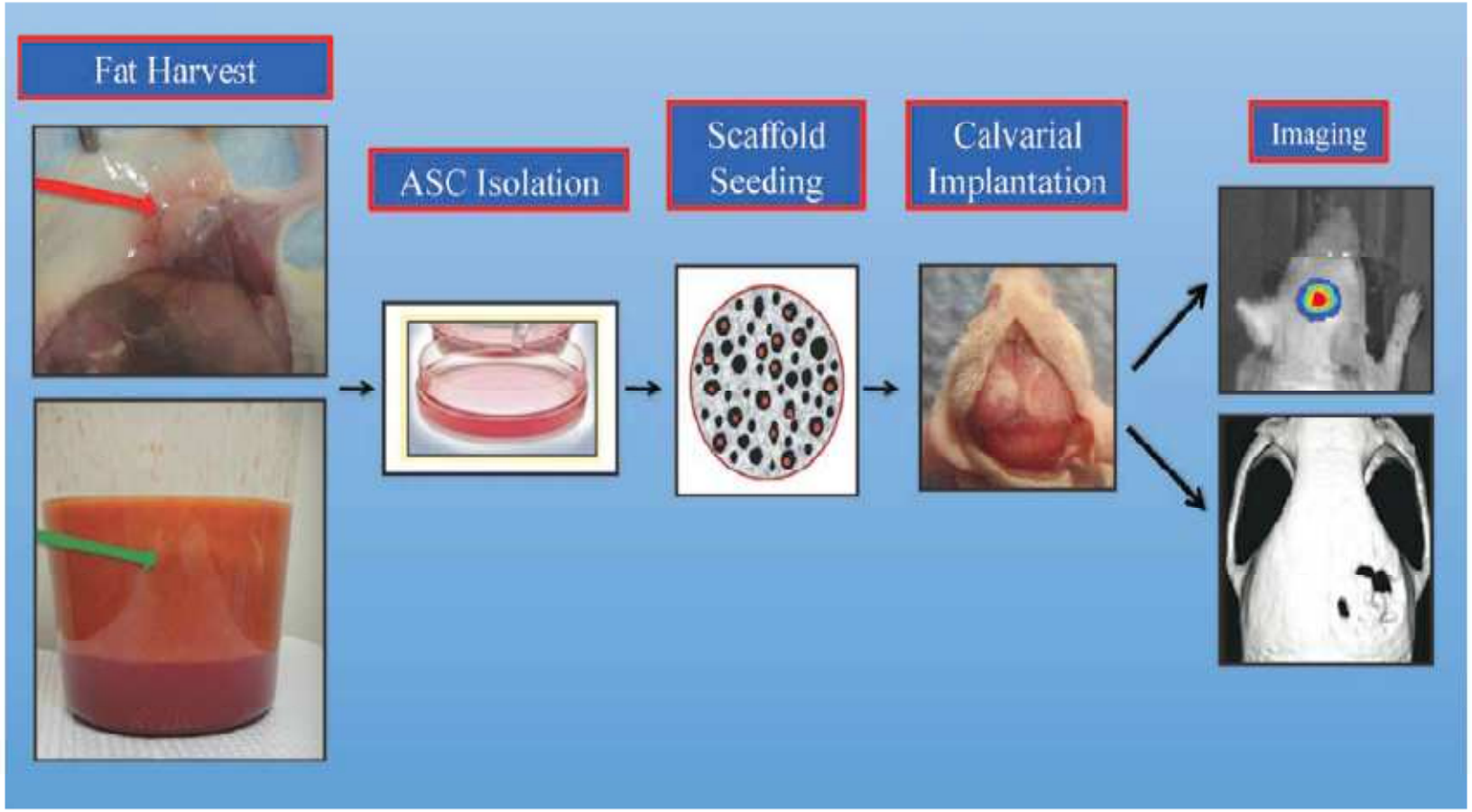


Figure 1. In vivo model for skeletal tissue engineering. Superior view of mouse left inguinal fat pad (left, top). Red arrow points to the fat pad which is dissected and subsequently digested for ASC harvest. Human lipoaspirate in sterile collection canister settled into two layers (left, bottom). The upper yellow layer (green arrow) is the adipose tissue from which ASCs are harvested. Subsequently, ASCs are isolated in vitro. A total of 150,000 cells can then be seeded on an osteoconductive scaffold (middle) and then the ASC-laden scaffold can be placed inside a critical sized calvarial defect in syngeneic for mASCs or nude athymic mice for hASCs (right). Mice can then be imaged using IVIS systems to detect cell viability and MicroCT to quantify osseous healing (right). Abbreviation: ASC, Adipose-derived stromal cell.

B. Levi, M.T. Longaker. Concise review: adipose-derived stromal cells for skeletal regenerative medicine. *Stem Cells* 2011; 29: 576-582.

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Table 1 Clinical trials using ASCs reported at clinicaltrials.gov as of May, 2010

Trial	Study phase	Condition	Locations
Study of Autologous Fat Enhanced w/ Regenerative Cells Transplanted to Reconstruct Breast Deformities After Lumpectomy (RESTORE-2)	IV, Active, not recruiting	Breast Neoplasms Carcinoma; Ductal; Breast Mammoplasty Mastectomy; Segmental, Lumpectomy; Breast Reconstruction	Brussels, Belgium; Florence, Italy; Madrid, Spain; Valencia, Spain; Glasgow, United Kingdom
Efficacy and Safety of Adipose Stem Cells to Treat Complex Perianal Fistulas Not Associated to Crohn's Disease (FATT1)	III, Completed	Anal Fistula	Mannheim, Germany; Madrid, Spain; Zaragoza, Spain; Pamplona, Spain; Tarrasa, Spain; Oxford, United Kingdom
Safety and Efficacy of Autologous Cultured Adipocytes in Patient With Depressed Scar	II/III, Completed	Depressed Scar	Seoul, Republic of Korea
Autologous Stem Cells Derived From Lipoaspirates for the Non-Surgical Treatment of Complex Perianal Fistula	II, Active, not recruiting	Perianal Fistula	Madrid, Spain
Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Type 2 Diabetics	I/II, Active, not recruiting	Type 2 Diabetes Mellitus	Makati City, Philippines; Quezon City, Philippines
Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Patients With Type 1 Diabetes	I/II, Recruiting	Type 1 Diabetes Mellitus	Makati City, Philippines; Quezon City, Philippines

B. Lindroos, R. Suurinen, S. Miettinen. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev and Rep*, 7:269-291. 2011.

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Autologous Mesenchymal Stem Cells From Adipose Tissue in Patients With Secondary Progressive Multiple Sclerosis (CMM/EM/2008)	I/II, Recruiting	Secondary Progressive Multiple Sclerosis	Sevilla, Spain
Allogenic Stem Cells Derived From Lipoaspirates for the Treatment of Recto-vaginal Fistulas Associated to Crohn's Disease (ALOREVA)	I/II, Recruiting	Rectovaginal Fistula; Crohn Disease	Madrid, Spain
Intraarterial Infusion of Autologous Mesenchymal Stem Cells From Adipose Tissue in Diabetic Patients With Chronic Critical Limb Ischemia (CeTMAd/ICPD200)	I/II, Suspended	Chronic Critical Limb Ischemia	Sevilla, Spain
A Randomized Clinical Trial of AdiPOse-Derived Stem ceLLs in the Treatment of Patients With ST-Elevation myOcardial Infarction - The APOLLO Trial	I, Active, not recruiting	Myocardial Infarction; Coronary Arteriosclerosis; Cardiovascular Disease; Coronary Disease	Rotterdam, Netherlands; Madrid, Spain
A Randomized Clinical Trial of adiPose-deRived stEm & Regenerative Cells In the Treatment of Patients With Non revaScularizable ischEmic Myocardium - The PRECISE Trial	I, Active, not recruiting	Ischemic Heart Disease; Coronary Arteriosclerosis; Cardiovascular Disease; Coronary Disease; Coronary Artery Disease	Copenhagen, Denmark; Rotterdam, Netherlands; Utrecht, Netherlands; Madrid, Spain
Safety and Efficacy Study of Autologous Cultured Adipose -Derived Stem Cells for the Crohn's Fistula	I, Active, not recruiting	Crohn's Fistula	Seoul, Republic of Korea

B. Lindroos, R. Suurinen, S. Miettinen. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev and Rep*, 7:269-291. 2011.

Autologous Adipose-Derived Stem Cell Transplantation in Patients With Lipodystrophy (AADSCTPL)	I, Recruiting	Lipodystrophy	Porto Alegre, Brazil
Liver Regeneration Therapy by Intrahepatic Arterial Administration of Autologous Adipose Tissue Derived Stromal Cells	I, Recruiting	Liver Regeneration Therapy	Kanazawa, Japan
Safety Study of Autologous Cultured Adipose -Derived Stem Cells for the Fecal Incontinence	I, Recruiting	Fecal Incontinence	Seoul, Republic of Korea
Long-term Safety and Efficacy of Adipose-derived Stem Cells to Treat Complex Perianal Fistulas in Patients Participating in the FATT-1 Randomized Controlled Trial (LTE)	Recruiting	Perianal Fistulas	Terrasa, Spain; Cantabria, Spain; Madrid, Spain; Valencia, Spain
Liver Regeneration Therapy Using Autologous Adipose Tissue Derived Stromal Cells	Recruiting	Liver Cirrhosis	Kanazawa, Japan
Mesenchymal Stromal Cells Secreting Neurotrophic Factors (MSC-NTF), in Patients With Amyotrophic Lateral Sclerosis (ALS)	Not yet recruiting	Amyotrophic Lateral Sclerosis	Jerusalem, Israel

B. Lindroos, R. Suurinen, S. Miettinen. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev and Rep*, 7:269-291. 2011.

In vivo use of AT-MSC: contradictory results

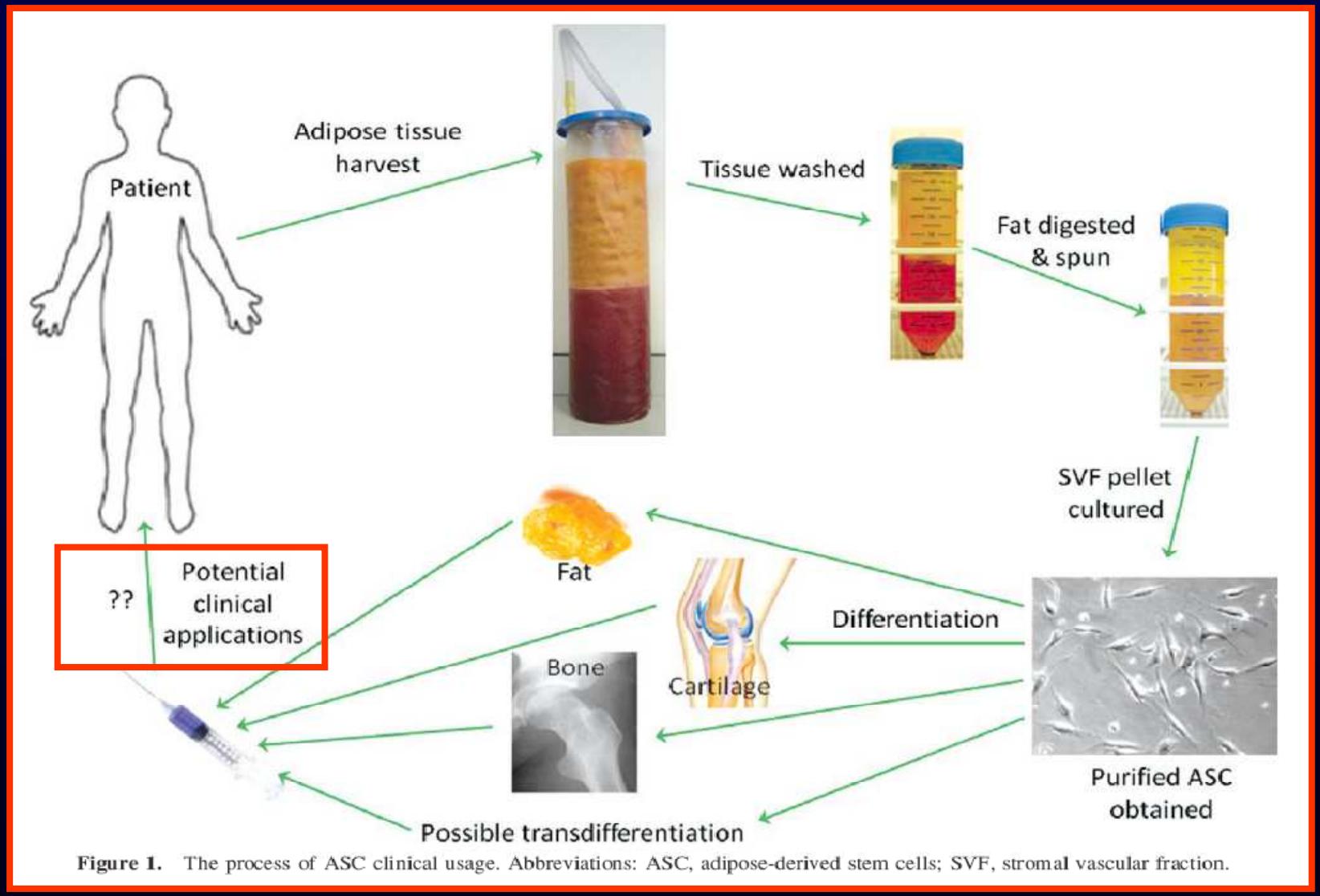

Figure 1. The process of ASC clinical usage. Abbreviations: ASC, adipose-derived stem cells; SVF, stromal vascular fraction.

M. Locke, V. Feisst, P.R. Dunbar. Concise Review: Human Adipose-Derived Stem Cells: Separating Promise from Clinical Need. *Stem Cells* 2011; 29: 404–411.

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In vivo use of AT-MSC: contradictory results

Table 1. Summary of data from reviewed papers

Reference	Cell composition	Differentiation and inducers	Histochemical analysis	Gene marker analysis	Clinical outcome
[17, 18]	50%human SVF 50% human-lipoaspirate	None	None	None	Improved fat grafting outcomes (face and breast)
[20]	Human SVF	None	None	None	Healing of radiation skin damage and osteoradionecrosis.
[25]	Culture purified human ASC	Adipogenic: IBMX/dexamethasone/insulin/indomethacin Osteogenic: vitamin D3/ascorbate-2-phosphate/β-glycerophosphate	Adipogenic: Nile red Osteogenic: BM Purple (ALP activity), alizarin red	Adipogenic mRNA: *PPARγ/AP2* Osteogenic mRNA: *COL1A1/ALP/BGLAP*	—
[29]	Culture purified human ASC	Adipogenic, osteogenic and chondrogenic medium: Gibco BRL	Adipogenic: oil red O Osteogenic: Alizarin red, ALP assay Osteogenic: Alizarin red, ALP assay Osteogenic: Alizarin red, ALP assay	Osteogenic mRNA: *RUNX2/BGLAP/SPP1/COL1A1*	—
[30]	Murine SVF	None	H&E	None	Healing of bone defects.
[31]	Murine ASC	Adipogenic: IBMX/dexamethasone/insulin Osteogenic: Ascorbic acid/dexamethasone/β-glycerophosphate	Adipogenic: oil red O Osteogenic: Alizarin red	Osteogenic protein: *BGLAP*	Formation of bone and adipose tissue in vivo graft healing.
[32]	Human SVF + bone graft	None	None	None	Potential for improved bone.
[33]	Human ASC	Adipogenic: IBMX/dexamethasone/insulin/pantothenate Osteogenic:Ascorbic acid/dexamethasone/β-glycerophosphate Chondrogenic: TGFβ	Adipogenic: oil red O Osteogenic: ALP assay Chondrogenic: Alcian blue	Osteogenic mRNA: *ALP/RUNX2/BGLAP/SPP1/COL1A1*	Case study of bone defect healing.
[39]	Culture purified human ASC	Chondrogenic: Dexamethasone/ascorbate-2-phosphate/TGFβ2 /BMP7	Chondrogenic: Safranin O	Chondrogenic mRNA and protein: *COL1A1/COL2A1/COL10A1/RUNX2* mRNA only *SOX9*	—
[41]	Culture purified rabbit ASC	Myogenic: 5-azacytidine	None	None	Reconstitution of osteochondral defects.
[42]	Canine SVF	None	None	None	Improvement in observed lameness.
[43]	Culture purified human ASC	None	None	None	Rheumatoid arthritis symptom suppression.
[44]	Culture purified human ASC	None	None	None	Suppression of immune responses.

Abbreviations: ALP, alkaline phosphatase; ASC, adipose-derived stem cell; BGLAP, osteocalcin; BMP7, bone morphogenetic protein 7; COL1A1, type I collagen; IBMX, 3-isobutyl-1-methylxanthine; PPARγ, peroxisome proliferator-activated receptor gamma; RUNX2, runt-related transcription factor 2; SPP1, osteopontin; SVF, stromal vascular fraction; TGFβ2, transforming growth factor beta 2.

M. Locke, V. Feisst, P.R. Dunbar. Concise Review: Human Adipose-Derived Stem Cells: Separating Promise from Clinical Need. *Stem Cells* 2011; 29: 404–411.

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The burgeoning of human ASC research may appear to indicate that we are far advanced in our understanding of ASC and our ability to manipulate them. However, the current literature is often difficult to interpret due to uncertainty about the cells being used, that is, whether pure ASCs or mixtures of cells including ASCs, and the use of assays of uncertain significance to "confirm" differentiation down particular lineages. Methods used to differentiate ASC down these different lineages are still relatively generic and unsophisticated in many publications, and a lack of control cells such as differentiated mesenchymal cells can make it difficult to determine whether "differentiation" observed is unique to ASC or a property shared with other mesenchymal cells. The field would clearly benefit from more standardized disclosure of the experiments conducted, as proposed in Figure 2. Such dis-

M. Locke, V. Feisst, P.R. Dunbar. Concise Review: Human Adipose-Derived Stem Cells: Separating Promise from Clinical Need. *Stem Cells* 2011; 29: 404–411.

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In vivo use of AT-MSC: method standardization

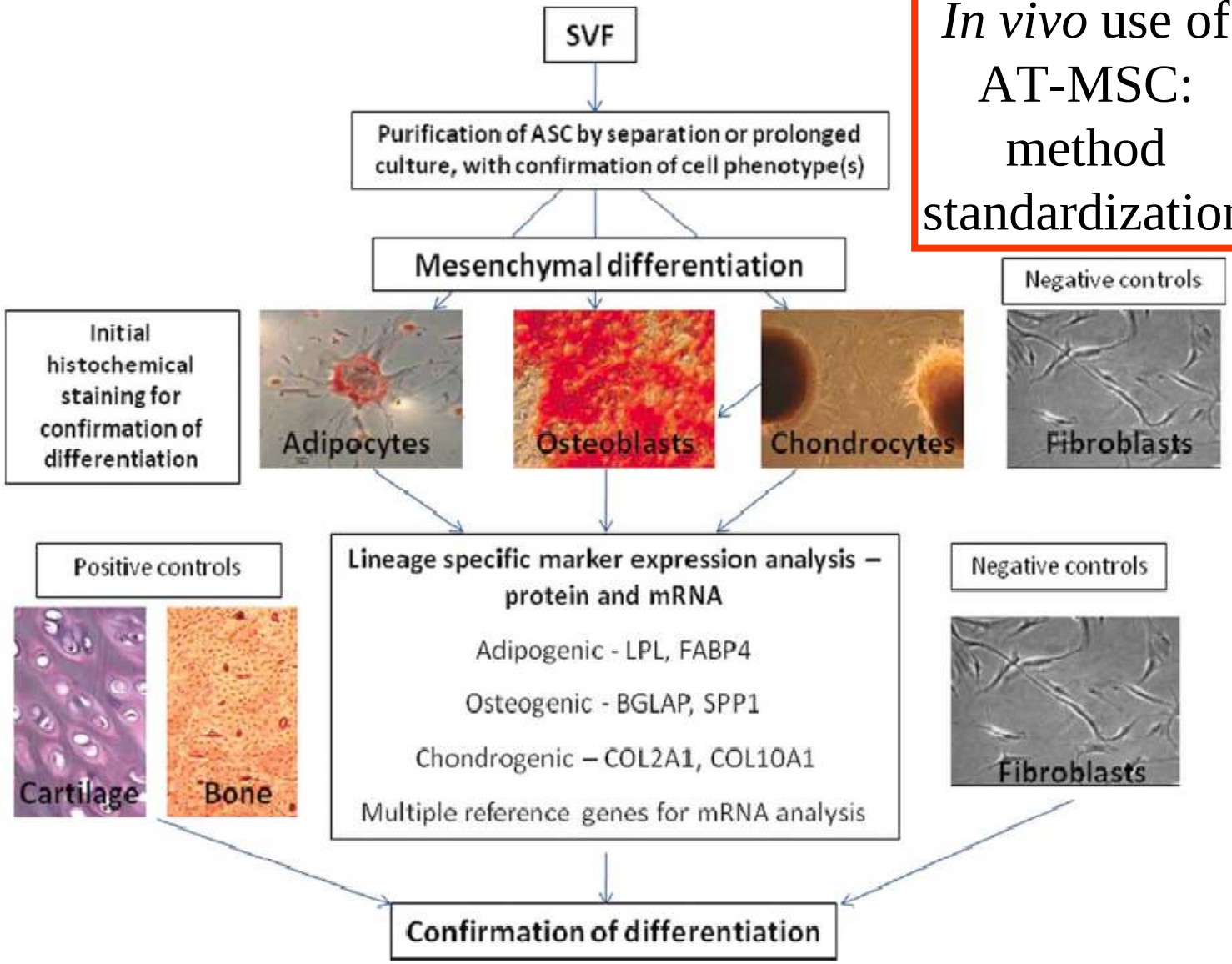

Figure 2. The schematic representation of the proposed "minimal information set" required to confirm ASC differentiation. Abbreviations: ASC, adipose-derived stem cells; BGLAP, osteocalcin; FABP4, fatty acid binding protein 4; LPL, lipoprotein lipase; SPP1, osteopontin; SVF, stromal vascular fraction; COL10A1, type X collagen; COL2A1, type II collagen.

M. Locke, V. Feisst, P.R. Dunbar. Concise Review: Human Adipose-Derived Stem Cells: Separating Promise from Clinical Need. *Stem Cells* 2011; 29: 404–411.

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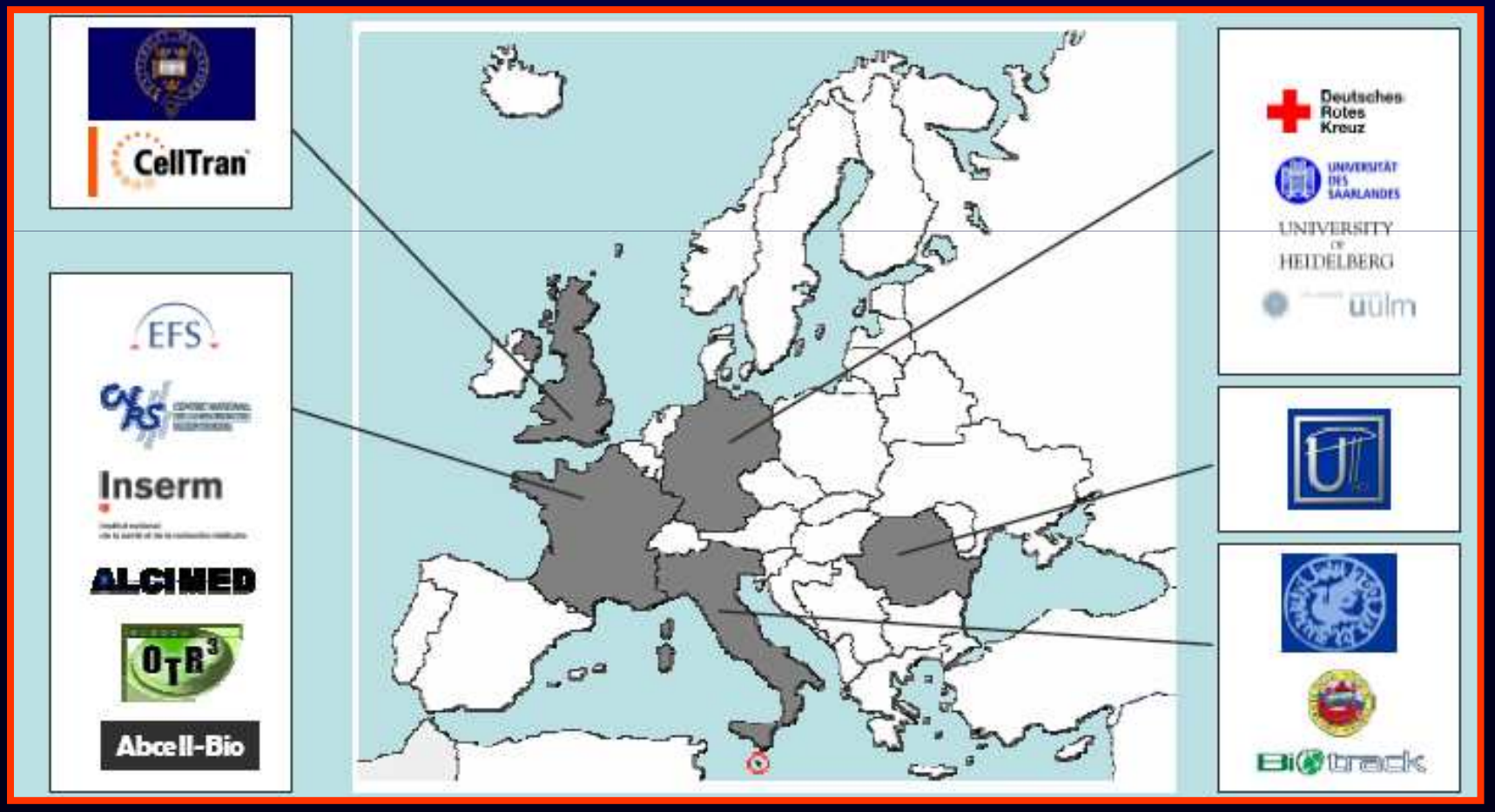
CASCADE

(Cultivated Adult Stem Cells as Alternative for Damaged tissuE)

Coordinator: Dr. Luc Sensebé, EFS, Toulouse, France



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CASCADE - Obiettivi

Primary Objective 1: Development of culture conditions and standard tools for the generation of allogeneic and autologous GMP-grade MSC derived from bone marrow (BM), adipose tissue (AT), umbilical cord blood (CB) and amniotic membrane (AM).

Intermediate objectives are:

1.1 to define serum-free media, materials and substances for culture surface functionality, carrier surface, cell isolation, amplification and differentiation techniques to generate MSC with defined quality and safety profiles;

1.2 to define protocols for the immuno-toxicological and mutagenesis safety assessment of GMP-processed MSC;

1.3 to define standard protocols for MSC characterisation, screening and banking;

1.4 to define quality standards for starting materials from BM, AT, CB, AM;

1.5 to assess the selection criteria for donors; traceability; laboratory tests for donor validations.

Primary Objective 2: Evaluation of the therapeutic potential and the mechanism of action of MSC for chronic wound healing of skin and cornea by *in vitro* and *in vivo* models.

Intermediate objectives are:

2.1 to evaluate the therapeutic potential and the mechanism of action of MSC for chronic wound healing of skin and cornea by in vitro models;

2.2 to improve re-vascularisation of damaged tissues and to limit scar formation through the definition of MSC/dermofibroblast - endothelial progenitor cell (EPC) interaction;

2.3 to compare the immunological phenotype of autologous and allogeneic MSC before or after culture in defined media.

2.4 to characterise the hostile microenvironment of chronic venous leg ulcers and to establish a standardised clinical setting for the treatment of chronic leg ulcers with MSC;

2.5 to determine homing and migratory capacities of stem cells by establishing safe and efficient labelling procedures for MSC and EPC in order to ensure their effectiveness in the clinical setting.

CASCADE - Obiettivi

Primary Objective 3: definition of the general ethical perspectives of cellular therapies, including such issues as donations of BM cells for the generation of "pluri-use allogeneic" MSC products, particularly in relation to safety and quality aspects for donors and patients.

Sub-objectives:

3.1 to discuss pre-clinical data with regulatory authorities to improve the delays of getting marketing authorisation for using MSC products for clinical trials.

3.2 to collect information about possible legal problems for clinical use of cellular therapeutics related to patients in the field of cell processing.

3.3 to give an overview about the current legal situation of cellular therapeutics in European countries involved in the CASCADE project, including topics such as traceability of MSC products, banking issues, etc.

3.4 to disseminate the knowledge generated within the European scientific community by holding international workshops on an annual basis.

3.5 to implement and disseminate standards on GMP grade MSCs.

Primary Objective 4: Preparation of study protocols for multicentre, prospective, double-blind and placebo-controlled trials to evaluate the safety and efficacy of MSC applications for the treatment of skin ulcers and corneal chronic ulceration.

Sub-objectives:

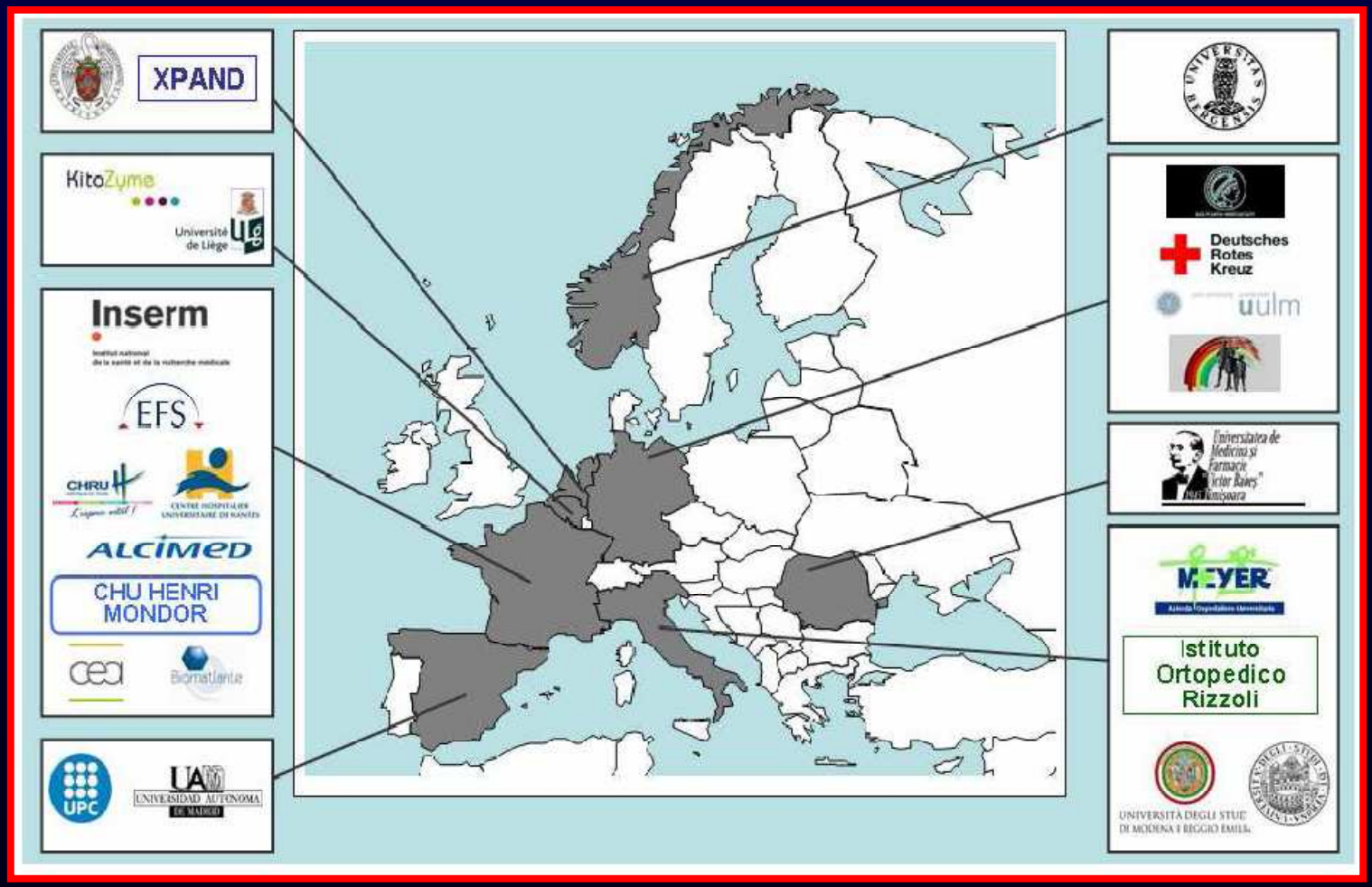
4.1 to design a study protocol to evaluate the efficacy of MSC grafts for patients with chronic non-healing venous leg ulcers;

4.2 to design a study protocol to evaluate the efficacy of MSC grafts for patients with chronic corneal ulcerations and delayed epithelial wound closure.

Progetto REBORNE - 7th FP EU 2008

(Regenerating Bone defects using New biomedical Engineering approaches)

Coordinator: Dr. Pierre Layrolle, INSERM, Nantes, France



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REBORNE - Obiettivi

Primary objective 1: Development of new biomaterials that trigger endogenous stem cells to differentiate into osteogenic lineages and rapidly promote bone healing.

Intermediate objectives are:

1.1 Developing new composite biomaterials with intrinsic osteoinductive property and high strength for load bearing applications as well as understanding the biological mechanisms of osteogenesis.

1.2 Collaborating for the development of hydrogels for percutaneous injection of cells and associated devices for minimal invasive surgery.

Secondary objective 2: Optimisation of safe and cost effective culture conditions, standard protocols and quality controls for the production of autologous and allogenic cells committed to osteogenesis with a predictable regenerative capability of a vascularised bone tissue.

Intermediate objectives are:

2.1 Evaluation of the potential of different sources of MSC regarding easiness of culture, cell yield, osteoblastic differentiation and safety.

2.2 Definition of the best way to culture the cells to obtain an optimal synergy between them and biomaterials promoting angiogenesis.

2.3 Development of new standardized potency assays combining MSC and biomaterials into cGMP settings.

2.4 Identification of new mesenchymal progenitors with robust osteogenic potential in biomaterials combination.

2.5 Demonstration of the safety and efficacy of allogenic stem cells both *in vitro* and *in vivo.*

%

REBORNE - Obiettivi

2.6 Development of quality control tests for MSCs from different sources and biomaterials in combination with MSCs, in particular for genotyping and phenotyping stability, potency and immunological properties.

2.7 Validation in pre-clinical animal models of the osteogenic potency of new biomaterials in combination with autologous or allogenic mesenchymal stem cells from different sources.

Third objective 3: Proving efficacy of regenerative medicine by using advanced biomaterials with or without cells in clinical trials.

Intermediate objectives are:

3.1 Evaluate the efficacy of autologous MSCs from bone marrow combined to biomaterial to obtain bone healing in patients with delayed consolidation in long bone dyaphyseal fractures.

3.2 Evaluate the efficacy of autologous MSCs from adipose tissue combined with biomaterial to obtain bone healing enhancement in adult patients with early avascular necrosis of the femoral head.

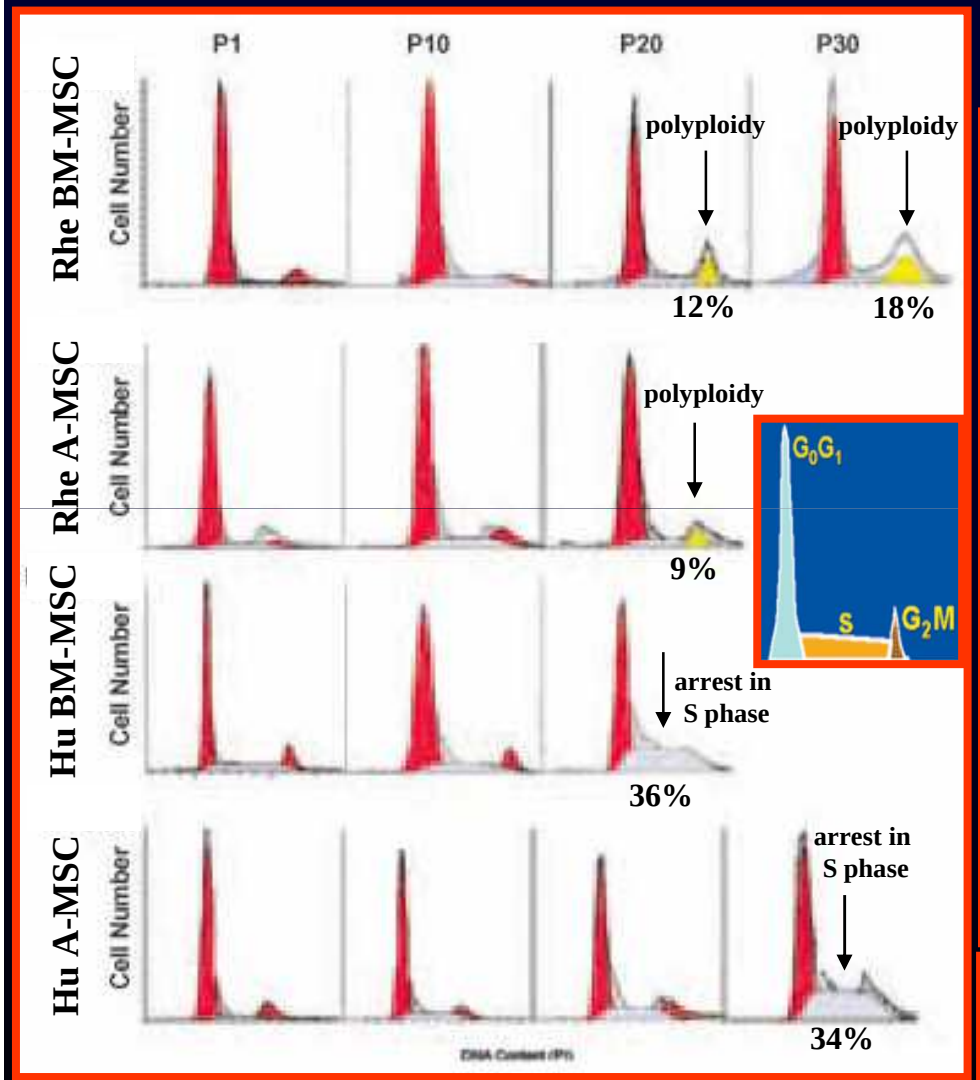
3.3 Enhance bone healing in young patients with avascular necrosis of the femoral condyle or head with previous haematological malignancy treated by immunosuppressive treatment, by a composite of biomaterial and allogenic MSCs from bone marrow, adipose tissue or cord blood.

3.4 Address bone reconstruction of cleft palates in children with injectable osteoinductive filler in combination or not with autologous MSCs from bone marrow.

3.5 Prove efficacy of biomaterials (MBCP granules and collagen membranes) and autologous MSCs injected post surgery for bone augmentation prior to dental implants.

3.6 Standardize new clinical approaches including imaging read-outs and serological assays as valuable tools in patients' follow-up after bone growth.

DIRECT NEOPLASTIC TRANSFORMATION OF BM-/AT-MSCs



Rhesus-MSC vs Human MSC (BM- vs Adipose-)

MSC type	Passage (3 donors)	Diploid cells (%)	Tetraploid cells (%)	Aneuploidy (%)*
rBMSC	P1	100%	0%	—
	P10	100%	0%	—
	P20	60%	40%	—
	P30	20%	70%	10%
	P90	20%	40%	40%
rASC	P1	100%	—	—
	P10	100%	—	—
	P20	90%	10%	—
hBMSC	P1	100%	—	—
	P10	100%	—	—
	P20	100%	—	—
hASC	P1	100%	—	—
	P10	100%	—	—
	P20	100%	—	—
	P30	100%	—	—

NOTE: The rest of the cells in that population were aneuploid.
*Aneuploidy includes the presence of a total chromosome number ranging from 55 to 81

Rhesus BMSCs showed 40% diploid metaphases (42, XY), whereas 20% metaphases had 84, XXYY karyotype at P90

Izadpanah R, et al. Long-term in vitro expansion alters the biology of adult MSCs.*Cancer Res 2008;68:4229-4238*

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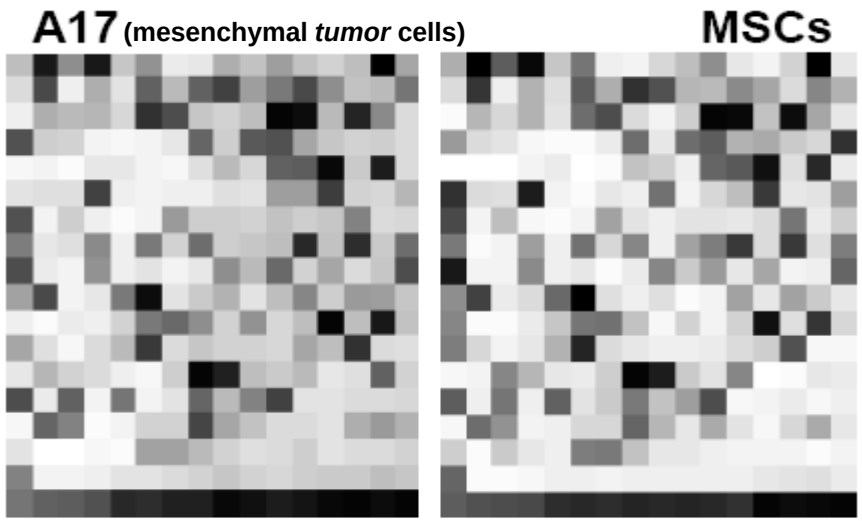
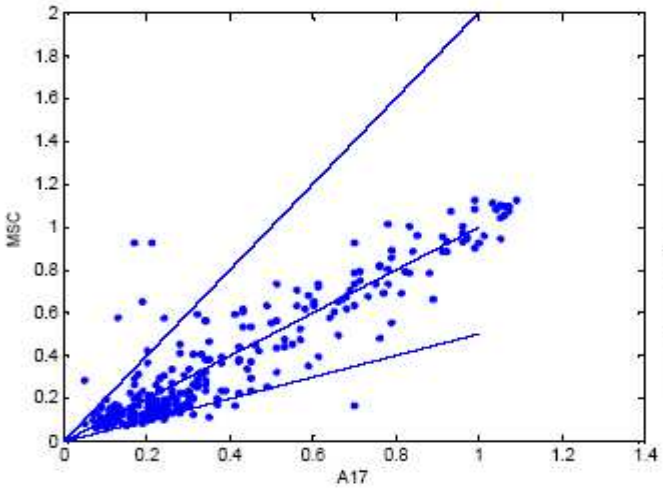
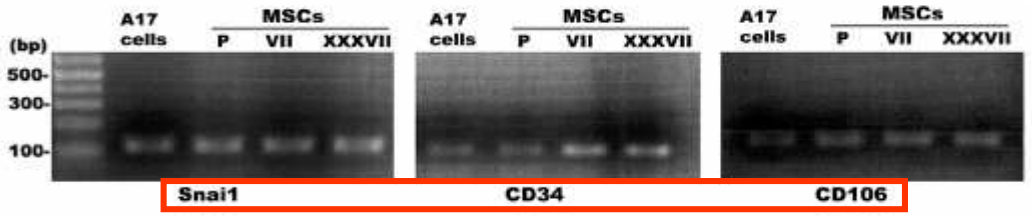
Mesenchymal tumour cells share the same gene pattern of AT-MSCs







M. Galiè, G. Konstantinidou, D. Peroni, I. Scambi, C. Marchini, P. Magnani, F. Merigo, M. Montani, F. Boschi, P. Marzola, R. Orrù, V. Lisi, M. Krampera, P. Farace, A. Sbarbati, A. Amici. Mesenchymal stem cells share molecular signature with mesenchymal tumor cells and favors the early tumor growth in syngeneic mice. *Oncogene 2008; 27:2542-2551*

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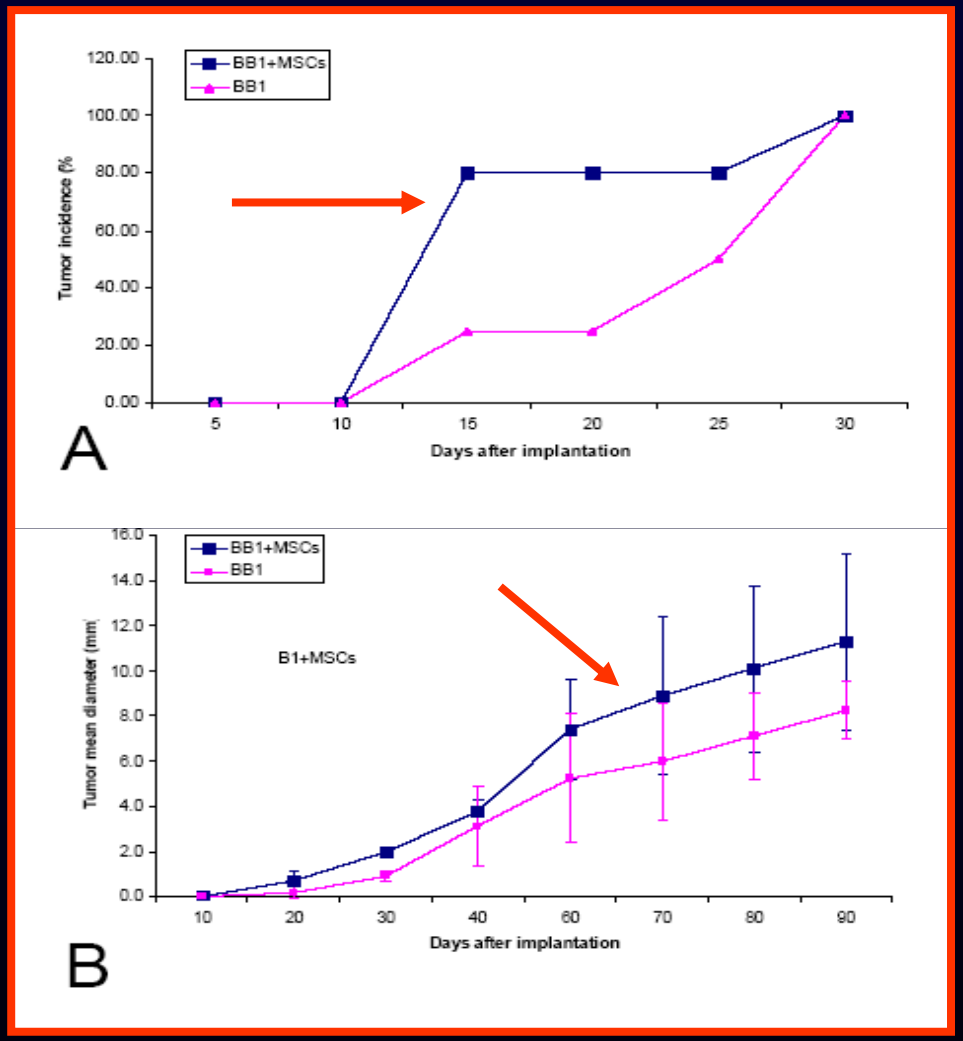
Co-implantation of AT-MSCs with BB1 cells (not spontaneous mammary carcinoma) in syngeneic mice



earlier tumor appearance

bigger size over a long-term follow-up

M. Galiè, G. Konstantinidou, D. Peroni, I. Scambi, C. Marchini, P. Magnani, F. Merigo, M. Montani, F. Boschi, P. Marzola, R. Orrù, V. Lisi, M. Krampera, P. Farace, A. Sbarbati, A. Amici. Mesenchymal stem cells share molecular signature with mesenchymal tumor cells and favors the early tumor growth in syngeneic mice. *Oncogene 2008; 27:2542-2551*

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Summary

- AT-MSC can be easily achieved by means of lipoaspirates or surgical procedures and can be efficiently expanded in culture
- AT-MSC and BM-MSC share similar immunophenotype (with the exception of a few markers, i.e. CD106), gene profile, differentiation potential and immune regulatory properties *in vitro* and *in vivo*.
- AT-MSC display high regenerative activity in ischemic tissues and immune regulatory activity in CNS autoimmune diseases; further studies are needed for their clinical use in GvHD
- safety controls must be similar to those for BM-MSC, including assays for neoplastic transformation and senescence

AT-MSC are promising tools for regenerative medicine

Grasso è bello, ed è pure staminale…..

Messico, super obeso esce di casa dopo cinque anni

Manuel Uribe non usciva di casa da cinque anni. La sua mezza tonnellata e oltre di peso glielo impediva. Ora è dimagrito 200 kg e si concede finalmente un giro all'aria aperta …. Quarant'anni, messicano, Uribe è stato tirato fuori di casa con una gru

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Laboratorio di Ricerca sulle Cellule Staminali

http://www.stemcellreslab-verona.it

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