Generation of mesenchymal stromal cells in the presence of platelet lysate: a phenotypical and functional comparison of umbilical cord blood- and bone marrow-derived progenitors

Maria Antonietta Avanzini,[1] Maria Ester Bernardo,[1] Angela Maria Cometa,[1] Cesare Perotti,[2] Nadia Zaffaroni,[3] Francesca Novara,[4] Livia Visai,[5] Antonia Moretta,[1] Claudia Del Fante,[2] Raffaella Villa,[3] Lynne M. Ball,[6] Willem E. Fibbe,[7] Rita Maccario,[1,8] and Franco Locatelli[1]

[1]Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Pavia, Italy; [2]Servizio di Immunoematologia e Medicina Trasfusionale, Banca del Cordone Ombelicale, Fondazione IRCCS Policlinico San Matteo, Pavia; [3]Dipartimento di Oncologia Sperimentale, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; [4]Dipartimento di Patologia Umana ed Ereditaria, Sezione Biologia Generale e Genetica Medica, Università di Pavia, Pavia; [5]Dipartimento di Biochimica and Center for Tissue Engineering (CIT), Università di Pavia, Pavia; [6]Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands; [7]Department of Immunohematology and Blood Transfusion, Center for Stem Cell Therapy, Leiden University Medical Center, Leiden, and [8]Cell Factory, Fondazione IRCCS Policlinico San Matteo, Pavia

Citation: Avanzini MA, Bernardo ME, Cometa AM, Perotti C, Zaffaroni N, Novara F, Visai L, Moretta A, Del Fante C, Villa R, Ball LM, Fibbe WE, Maccario R, and Locatelli F. Generation of mesenchymal stromal cells in the presence of platelet lysate: a phenotypical and functional comparison of umbilical cord blood- and bone marrow-derived progenitors Haematologica 2009; 95:xxx doi:10.3324/haematol.2009.006171

©2010 Ferrata Storti Foundation. This is an open-access paper.

©Ferrata Storti Foundation

Supplementary Table 1. Kinetics of cytokine production in culture supernatants.

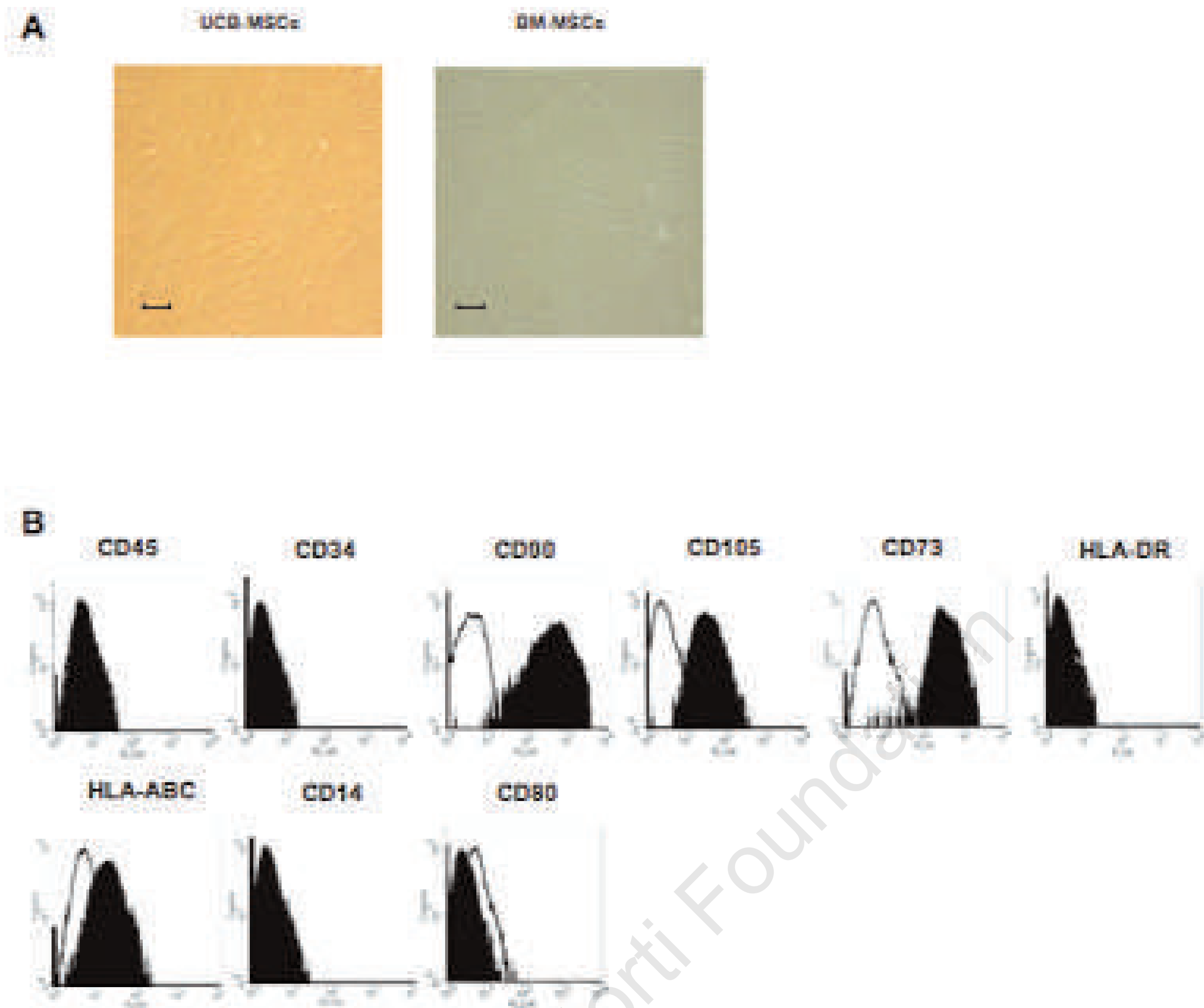
	12-h	24-h	48-h
IFNγ			
ctrl-MLC	2	6	107
MLC+UCB3-MSCs	1	4	77
MLC+UCB6-MSCs	4	9	71
IL-10			
Ctrl-MLC	9	11	16
MLC+UCB3-MSCs	8	20	17
MLC+UCB6-MSCs	10	20	20
IL-6			
Ctrl-MLC	868	1029	1000
MLC+UCB3-MSCs	44,000	41,000	44,000
MLC+UCB6-MSCs	48,000	45,000	46,000

Concentrations of IFN-γ, IL-10, IL-6 were quantified in MLC supernatants collected after 12, 24, 48-hour (-h) culture in the absence (ctrl-MLC) or presence of UCB3-MSCs (MLC+UCB3-MSCs) and UCB6-MSCs (MLC+UCB6-MSCs). Results are reported as pg/ml. IFNγ, IL-10 were undetectable in the supernatants of UCB-MSCs simultaneously cultured in the absence of PBMCs. Both UCB3- and UCB6-MSCs were able to constitutively secrete IL-6 in culture supernatants (peak of constitutive secretion at 48 hours was 2,425 pg/ml and 4,569 pg/mL, respectively).

Supplementary Table 2. Constitutive expression of HLA-G in UCB-derived and BM-derived MSCs at passage 3.

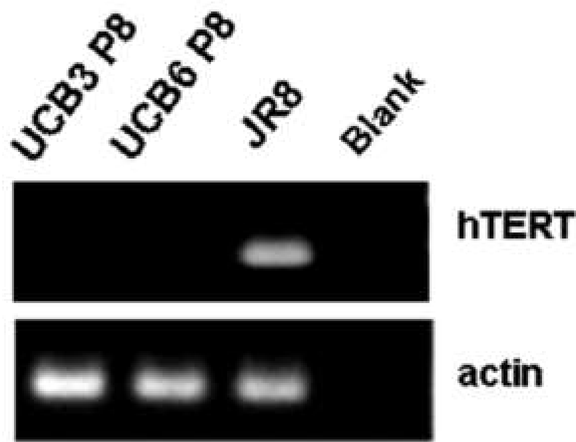
	mHLA-G %	MFI-R	iHLA-G %	MFI-R	sHLA-G U/mL
Exp 1					
UCB3-MSCs	73	2.8	100	11.2	31
BM1-MSCs	10	3.4	100	14.2	30
Exp 2					
UCB6-MSCs	78	4.3	100	10.2	30
BM2-MSCs	31	3.6	98	11.0	49

mHLA-G = membrane HLA-G; iHLA-G: intracellular HLA-G; sHLA-G: soluble HLA-G; %: percent of positive cells; MFI-R: mean fluorescence intensity ratio. Soluble HLA-G levels are expressed in U/ml. Two independent experiments are presented, in which UCB3-MSCs and BM1-MSCs from donor 227 (Exp 1) and UCB6-MSCs and BM2-MSCs from donor 527 (Exp 2) were tested.

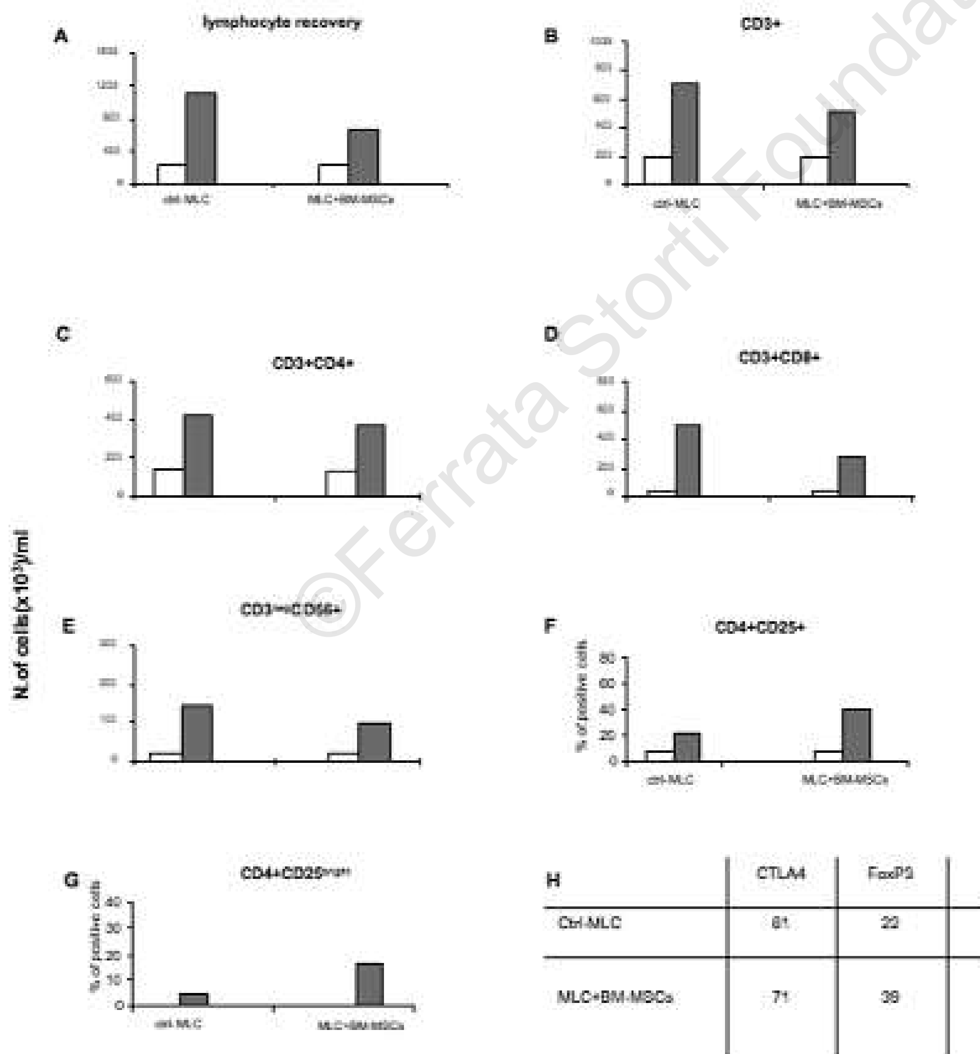


Supplementary Figure 1. A. A representative photograph of MSCs derived from UCB N.3 (UCB3-MSCs) at passage (P) 2, expanded in the presence of PL. UCB-MSCs display the typical spindle-shaped morphology, similar to that of BM-derived MSCs (BM-MSCs) cultured in 5% PL-supplemented medium (BM-MSCs from donor 2, Bernardo *et al.*[27] Magnification x10. Scale bar indicates 50 mm. B. Immunophenotypic characterization of UCB3-MSCs at P2 by flow cytometry. UCB-MSCs express CD90, CD73, CD105 and HLA-class I surface antigens, whereas they are negative for CD34, CD45, CD14, CD80 and HLA-DR.

©Ferrata Storti Foundation

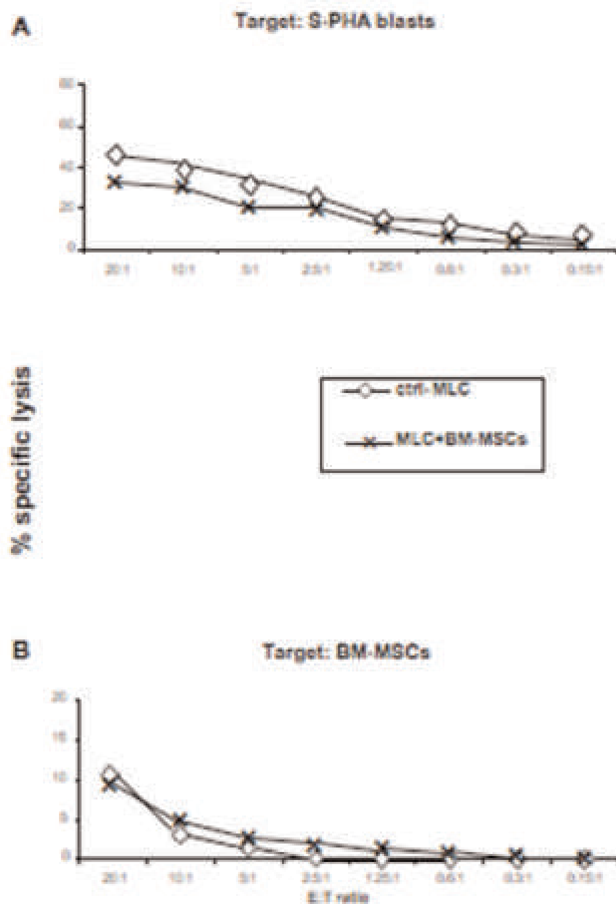


Supplementary Figure 2. Expression of h-TERT mRNA, as detected by RT-PCR in UCB3- and UCB6-MSC cultures at P8. b-actin was used as the external standard. The telomerase-positive cell line JR8 was used as a positive control. The blank represents a negative control to which no RNA was added.



©Ferrata Storti Foundation

Supplementary Figure 3. Effect of BM-MSCs, expanded in the presence of PL and previously reported27, on T and NK-lymphocyte subset expansion induced by allogeneic stimulus. Recovery of total number of lymphocytes (A), $CD3^+$ (B), $CD3^+CD4^+$ (C), $CD3^+CD8^+$ (D), $CD3^{neg}CD56^+$ NK cells (E), $CD4^+CD25^+$ (F), $CD4^+CD25^{bright}$ (G) T-lymphocyte subsets and with respect to the initial number (white columns), was assessed after 10-days primary culture (gray columns). Percentages of $CTLA4^+$ and $Foxp3^+$ cells were calculated on gated $CD4^+CD25^+$ T cells (H). MLC was performed in the absence (Ctrl-MLC) or presence of third-party BM-MSCs cultured in 5% PL (MLC+BM-MSCs). The MSCs were added at a responder (R)-PBMC/MSC ratio of 10:1; results are expressed as number of cells/mL of culture. The mean of two independent experiments (Exp 1, Exp 2) 27 is reported.



Supplementary Figure 4. Effect of third-party BM-MSCs, expanded in the presence of 5% PL and previously reported,[27] on cell-mediated cytotoxic activity induced by allogeneic stimulus. ^{51}Cr-labeled target cells included S-PHA (A) and the same lots of BM-MSCs (B) added to MLCs. Effector to target (E:T) ratios ranged between 20:1 and 0.15:1. Results are expressed as percent specific lysis of target cells. The mean of two independent experiments (Exp 1, Exp 2) 27 is reported.

©Ferrata Storti Foundation