

Research topic: The immune microenvironment and the infectious complications of allo-HSCT

Persistence of KIR^{neg} NK cells after haploidentical hematopoietic stem cell transplantation protects from human Cytomegalovirus infection/reactivation

Clara Di Vito^{1*}, Nicolò Coianiz¹, Michela Calvi^{1,2}, Sara Terzoli^{1,3}, Elisa Zaghi¹, Simone Puccio⁴, Alessandro Frigo^{1,2}, Jacopo Mariotti⁵, Chiara De Philippis⁵, Daniele Mannina⁵, Barbara Sarina⁵, Rossana Minerì⁶, Vu Thuy Khanh Le-Trilling⁷, Mirko Trilling⁷, Luca Castagna⁵, Stefania Bramanti⁵, Armando Santoro⁵, Domenico Mavilio^{1,2*}.

¹Unit of Clinical and Experimental Immunology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

²Department of Medical Biotechnologies and Translational Medicine (BioMeTra), University of Milan, Milan, Italy.

³Department of Biomedical Sciences, Humanitas University, Rozzano, Milan, Italy.

⁴Laboratory of Translational Immunology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

⁵Bone Marrow Transplant Unit, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

⁶Molecular Biology Section, Clinical Investigation Laboratory, IRCCS Humanitas Research Hospital, Milan, Italy.

⁷Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany.

*** Correspondence:**

Clara Di Vito

clara.di_vito@humanitasresearch.it

Domenico Mavilio

domenico.mavilio@unimi.it; domenico.mavilio@humanitas.it

Supplementary Material

Supplementary tables

Supplementary table 1. Statistical analysis of NR and R patient characteristics.

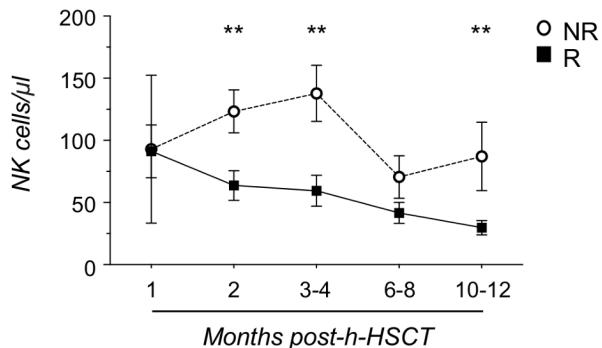
	NR (n; %)	R (n; %)	P value (χ^2)
Donor M	6; 60%	12; 60%	1
Donor F	4; 40%	8; 40%	
Recipient M	7; 70%	13; 65%	0.78
Recipient F	3; 30%	7; 35%	
Donor HCMV -	7; 70%	6; 30%	0.37
Donor HCMV +	3; 30%	14; 70%	
Recipient HCMV -	8; 80%	4; 20%	0.001***
Recipient HCMV +	2; 20%	16; 80%	
Acute GvHD YES	6; 60%	12; 60%	0.69
Acute GvHD NO	4; 40%	8; 40%	
Chronic GvHD YES	1; 10%	3; 15%	
Chronic GvHD NO	9; 90%	17; 85%	0.70
<hr/>			
	NR (median; range)	R (median; range)	P value (Mann-Whitney test)
Donor age	51; 29-62	37.5; 25-64	0.027*
Recipient age	33.5; 20-49	44; 20-70	0.053

Abbreviations: NR, not-reactivated patients; R, reactivated patients; F, Female; M, Male; GvHD, Graft versus Host Disease.

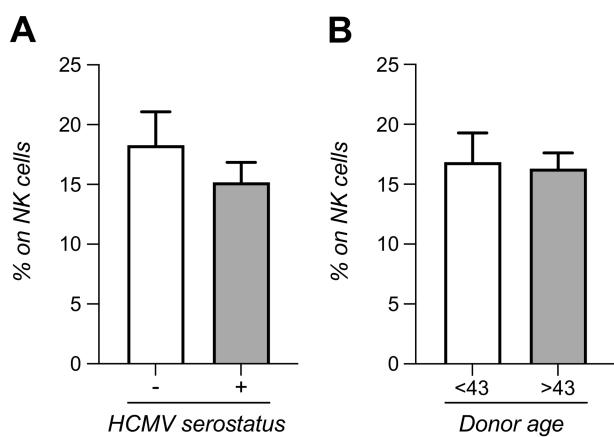
Supplementary table 2. Characteristics of patients used in *in vitro* infection experiments.

Group	Day post-h-HSCT used for in vitro infection experiments	HCMV d/r	HCMV- I/R (day post-h-HSCT)			Sex: d/r	r: age	d: age	Disease; status before transplant	Follow-up (weeks)
NR	369	+/+	NA	M/M	25	51	NHL; CR	53		
NR	231	+/-	NA	M/F	26	62	HL; CR	51		
NR	228	+/+	NA	F/M	54	55	NHL; SD	51		
NR	377	-/-	NA	M/M	68	32	MDS; CR	54		
R	331	+/+	33	F/M	47	38	HL; CR	47		
R	258	+/-	62	M/M	62	35	NHL; PR	50		
R	241	-/+	31	M/M	70	37	MDS; SD	52		
R	362	+/+	54	M/M	52	50	HL; CR	52		

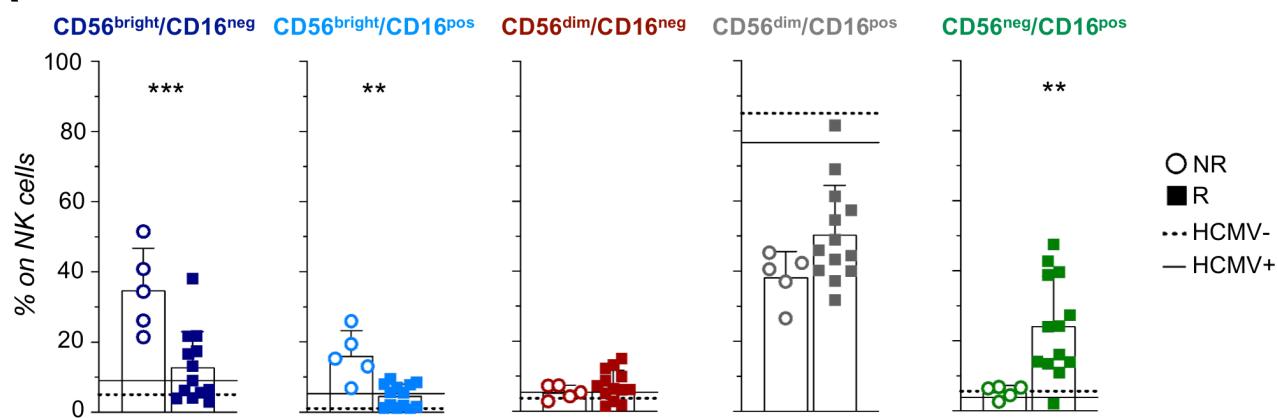
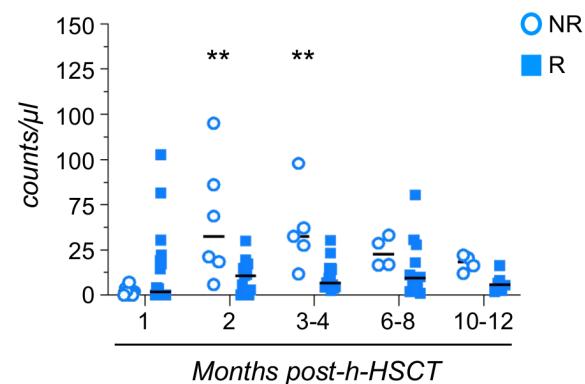
Abbreviations: NR, not-reactivated patients; R, reactivated patients; d, donor; r, recipient; F, Female; M, Male; NHL, Non-Hodgkin Lymphoma; HL, Hodgkin Lymphoma; MDS, Myelodysplastic Syndrome; CR, complete response; SD, stable disease; PR, partial response.

Supplementary Figures**Supplementary Figure 1. Longitudinal analysis of KIR^{neg} NK cell absolute counts in NR and R.**

Summary statistical graphs showing the absolute counts (NK cells/ μ l, mean \pm SD) of CD158b1b2j^{neg}/NKG2A^{pos}/NKG2C^{neg}/NKp30^{pos}/NKp46^{pos} NK cells in NR (○; n=10) and R (■; n=20) recipients at 1, 2, 3-4, 6-8, 10-12 months after the h-HSCT. Student's t-test NR vs R, **p<0.01.

**Supplementary Figure 2. KIR^{neg} NK cell frequency in donor grafts.**

Summary statistical graphs showing the frequency (%; mean \pm SD) of CD158b1b2j^{neg}/NKG2A^{pos}/NKG2C^{neg}/NKp30^{pos}/NKp46^{pos} NK cells on total NK cells in donor grafts subdivided based on (A) their HCMV serostatus or (B) age. The cut-off age of 43 corresponds to the median age of donors. Student's t-test, not significant.

A**B**

Supplementary Figure 3. NK cell subset distribution in recipients experiencing or not a HCMV infection/reactivation after h-HSCT.

(A) Summary statistical graphs showing the NK cell subset distribution (%; mean \pm SD) in NR (○; n=5) and R (■; n=20) recipients on total NK cells at 3-4 months after h-HSCT. Solid and dotted lines represent the average frequency of NK cell subsets in HCMV seropositive (HCMV+, n=5) and seronegative (HCMV-, n=5) healthy donors, respectively.

(B) Summary statistical graphs showing the absolute counts (cells/ μ L, line at mean) of circulating CD56^{bright}/CD16^{pos} NK cells in recipients either experiencing (R: ■; n=20) or not (NR: ○; n=10) HCMV reactivation/infection at different time points after h-HSCT.

Student's t-test NR vs R, *p<0.05, **p<0.01, ***p<0.001.