

Single-cell profiling reveals the dynamics of cytomegalovirus-specific T cells in haploidentical hematopoietic stem cell transplantation

Jasper J. P. van Beek,^{1*} Alessandra Roberto,^{1*} Simone Puccio,¹ Federica De Paoli,¹ Giulia Graziano,² Elisa Salviato,² Giorgia Alvisi,¹ Veronica Zanon,¹ Alice Scarpa,¹ Elisa Zaghi,¹ Michela Calvi,¹ Clara Di Vito,³ Rossana Mineri,¹ Barbara Sarina,¹ Chiara De Philippis,¹ Armando Santoro,^{1,4} Jacopo Mariotti,¹ Stefania Bramanti,¹ Francesco Ferrari,^{2,5} Luca Castagna,¹ Domenico Mavilio^{1,3} and Enrico Lugli¹

¹IRCCS Humanitas Research Hospital, Rozzano, Milan; ²IFOM, the FIRC Institute of Molecular Oncology, Milan; ³Department of Medical Biotechnologies and Translational Medicine (BioMeTra), University of Milan, Milan; ⁴Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan and ⁵IGM-CNR, Institute of Molecular Genetics "Luigi Luca Cavalli Sforza", National Research Council, Pavia, Italy

*JJPvB and AJ contributed equally as co-first authors

Correspondence: ENRICO LUGLI - enrico.lugli@humanitasresearch.it

Table S1. Patient characteristics and treatment.

ID	D/R Sex	D/R Age	Pathology	Conditioning	Graft type	GVHD prophylaxis ^f	aGVDH grade	Steroid therapy	CMV D/R	CMV viremia	CMV viremia onset	Antiviral treatment	Blood samples analyzed
#4	M/F	52/22	HL	Baltimore ^a	BM	FK	2	Yes	-/+	subclinical	day 57	Ganciclovir	day 56, 99, 126, 180, 386
#5	F/M	64/53	NHL	Baltimore ^a	BM	FK	No	No	+/+	subclinical	day 30	No	day 44, 57, 63
#8	F/F	61/33	HL	Baltimore ^a	BM	FK	No	No	+/+	subclinical	day 49	No	day 28, 74, 88, 112
#18	M/M	51/25	NHL	Baltimore ^a	BM	FK	1	No	+/+	No	NA	No	day 21, 29, 36, 43, 68, 75, 127, 369
#19	F/M	38/57	HL	Baltimore ^a	BM	FK	1	No	+/+	subclinical	day 33	No	day 29, 36, 43, 50, 68, 92, 113
#25	M/M	35/62	NHL	Baltimore ^a	BM	FK	2	No	+/-	clinical	day 55	Ganciclovir	graft and day 28, 42, 49, 58, 97, 121, 216, 349
#26	F/F	49/42	NHL	Baltimore ^a	BM	FK	1	No	+/+	subclinical	day 48	No	day 44, 51, 69, 76, 153, 202, 331
#34	F/M	63/33	AML	TBF MAC ^b	BM	FK	No	No	+/+	clinical	day 40	Ganciclovir	day 21, 29, 40, 96, 124, 190, 363
#38	F/M	27/35	ALL	TBF MAC ^b	BM	CsA	No	No	+/+	clinical	day 32	Ganciclovir	graft and day 28, 42, 74, 102, 144
#47	M/F	45/47	NHL	Baltimore ^a	BM	CsA	2	Yes	+/+	clinical	day 50	Ganciclovir	graft and day 28, 76
#52	M/M	53/38	HL	RIC ^c	BM	CsA	2	Yes	+/-	clinical	day 32	Valganciclovir	graft and day 22, 28, 67, 112, 182, 330
#56	F/F	29/28	HL	GITMO ^d	BM	CsA	No	No	+/+	clinical	day 14	Valganciclovir	graft and day 49, 83
#59	M/M	32/70	MDS	TBF RIC ^e	PBSC	CsA	No	No	+/+	clinical	day 32	Ganciclovir - Valganciclovir	graft and day 21, 36
#60	M/M	47/20	HL	Baltimore ^a	PBSC	CsA	2	Yes	-/+	clinical	day 39	Ganciclovir - Foscarnet	graft and day 21, 26, 64, 82, 141, 169, 348
#63	M/M	28/25	HL	Baltimore ^a	PBSC	CsA	No	No	+/-	clinical	day 41	Ganciclovir - Valganciclovir	graft and day 21, 27, 62, 100, 136, 161, 204, 282
#65	M/M	37/69	MDS	TBF RIC ^e	BM	CsA	1	No	-/+	clinical	day 31	Ganciclovir - Valganciclovir	day 21, 42, 59, 101, 126, 191, 302
#66	F/M	30/68	AML	TBF RIC ^e	PBSC	CsA	4	Yes	+/+	clinical	day 27	Ganciclovir	graft and day 38, 139
#70	F/M	40/36	ALL	TBF MAC ^b	PBSC	CsA	1	No	+/-	clinical	day 48	Valganciclovir	graft and day 48, 76, 104, 142, 167, 371
#76	M/F	33/56	HL	RIC ^c	BM	CsA	1	No	+/+	clinical	day 27	No	graft and day 90, 111, 223
#77	M/F	61/26	HL	Baltimore ^a	BM	CsA	1	No	+/-	No	NA	No	graft and day 27, 79, 121, 177, 205, 359
#84	M/M	25/52	MF	Baltimore ^a	PBSC	CsA	No	No	-/+	subclinical	day 95	No	graft and day 48, 62, 100, 146, 184, 219

^aSee Luznik et al.¹^bTBF MAC: thiotepa 10 mg/kg, busulfan 3.6 mg/kg, fludarabine 150 mg/m²^cRIC: thiotepa 10 mg/kg, cyclophosphamide 30 mg/kg, fludarabine 60 mg/m²^dGITMO: thiotepa 10 mg/kg, cyclophosphamide 60 mg/kg, fludarabine 60 mg/m²^eTBF RIC: thiotepa 5 mg/kg, busulfan 6.4 mg/kg, fludarabine 150 mg/m²^fAll patients received pt-cy and mycophenolate mofetil

ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; BM, bone-marrow; CsA, cyclosporine A; D, donor; F, female; FK, tacrolimus; HL, Hodgkin lymphoma; M, male; MAC, myeloablative conditioning; MDS, myeloid dysplastic syndrome; MF, myelofibrosis; NA, not applicable; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells; R, recipient; RIC, reduced intensity conditioning; TBF, thiotepa-busulfan-fludarabine

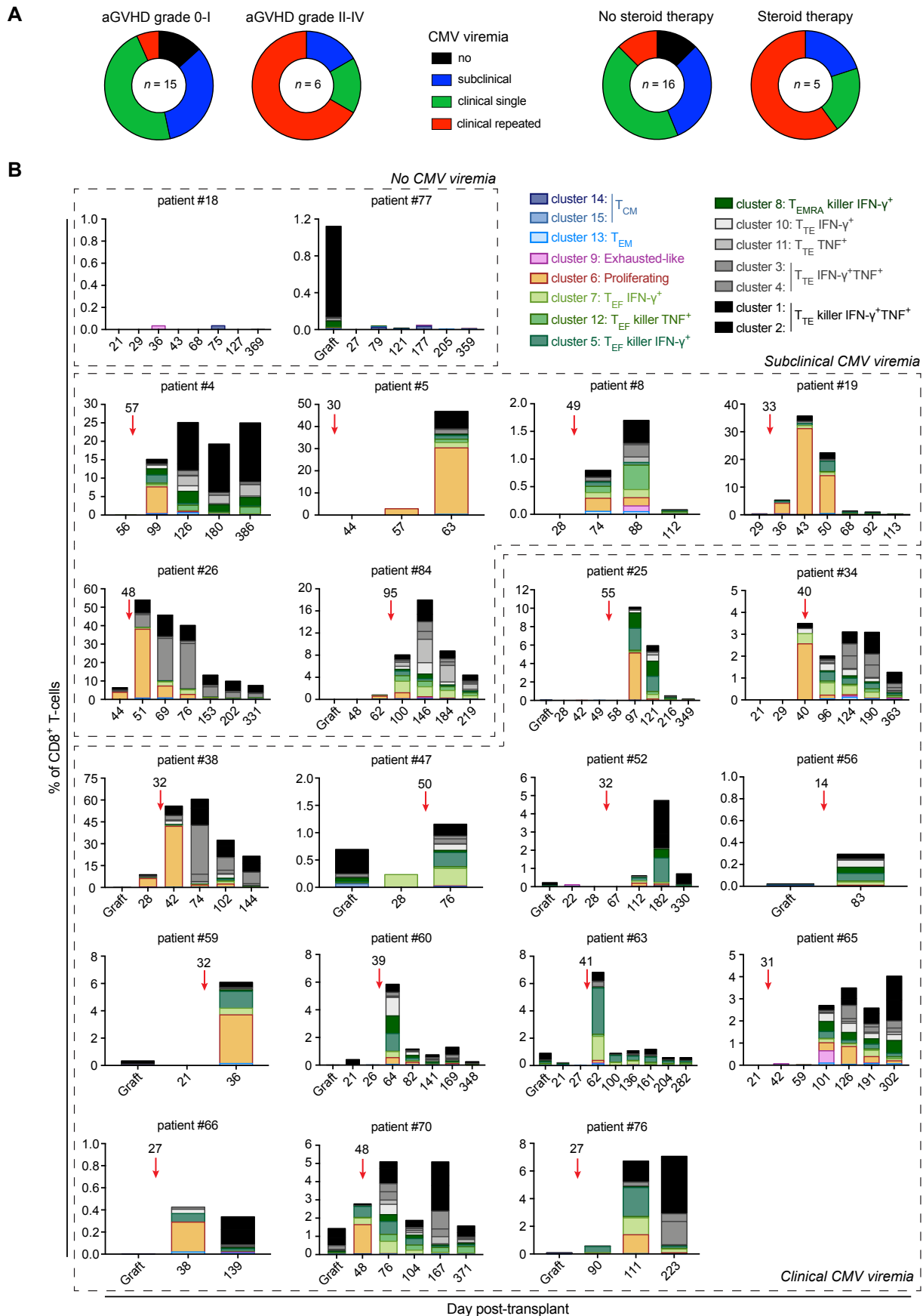


Figure S1. Impact of aGVHD and corticosteroid therapy on CMV reactivation, and longitudinal high-dimensional profiling of CMV-specific CD8⁺ T-cells in recipients of haplo-HSCT with pt-cy. **A)** CMV reactivation among recipients of haplo-HSCT with pt-cy, stratified by occurrence of aGVHD or corticosteroid therapy. **B)** Frequencies of CMV-specific CD8⁺ T-cell PhenoGraph clusters among total CD8⁺ T-cells are shown for all measured time points. Arrows indicate the day post-transplant of CMV viremia onset. Patients are grouped according to post-transplant CMV viral load.

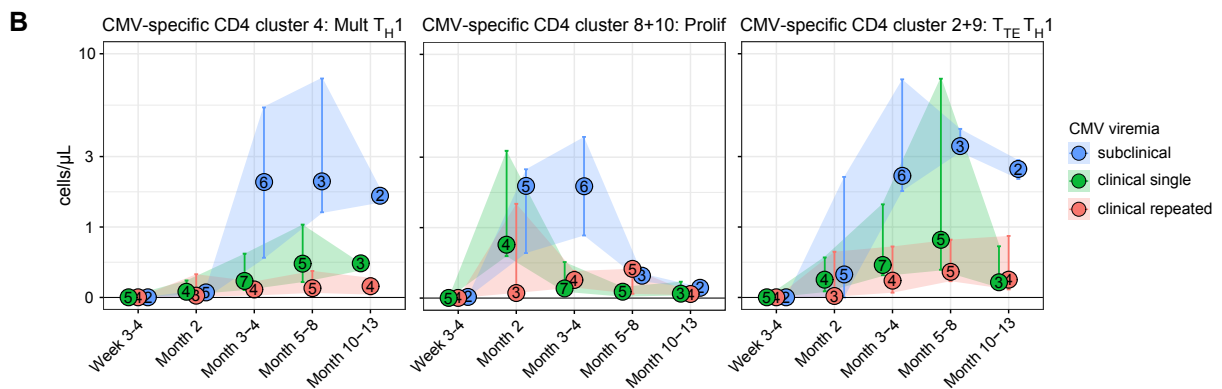
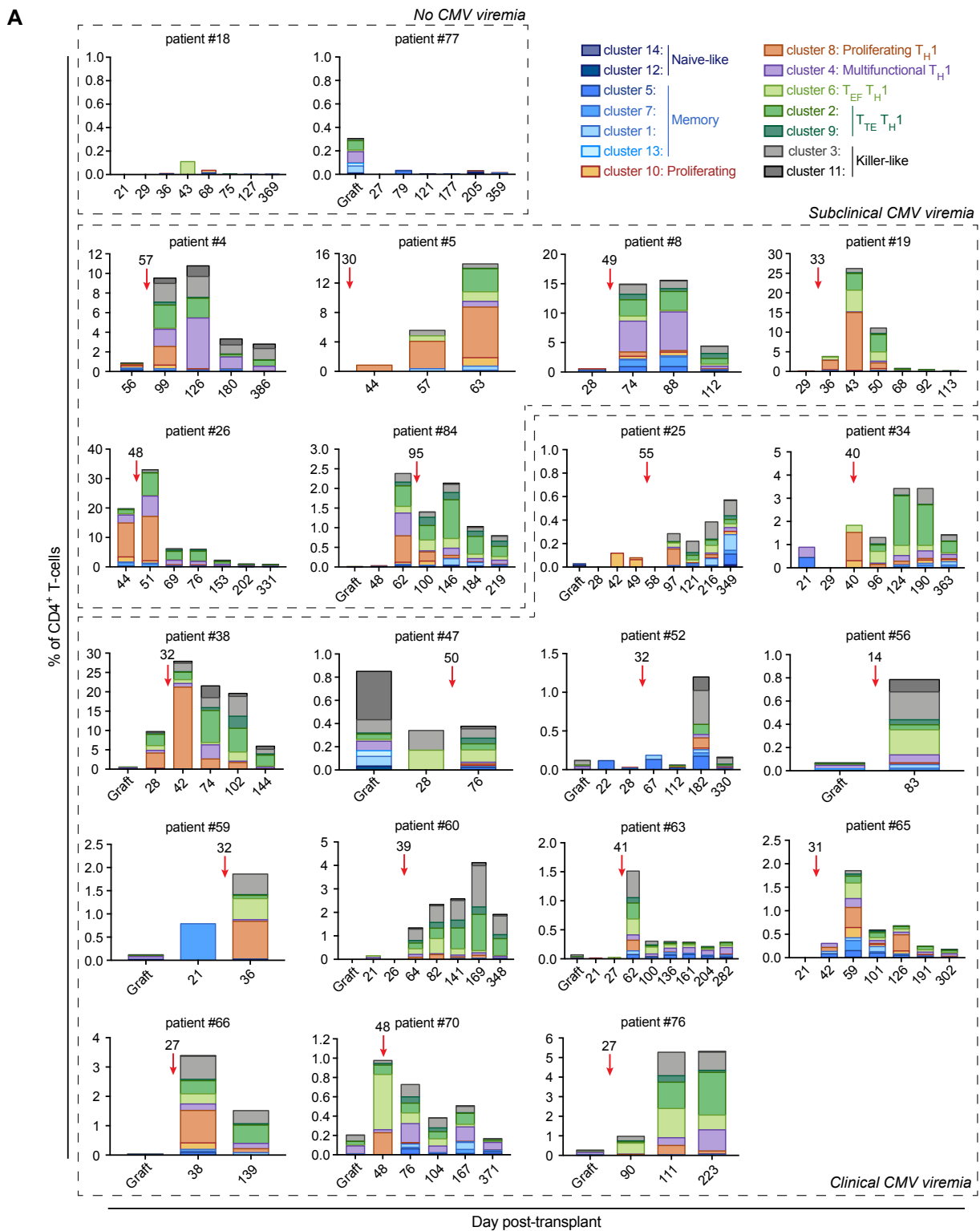


Figure S2. CMV viremia control following haplo-HSCT with pt-cy associates with the development of distinct CD4⁺ antigen-specific T-cell immunophenotypes. A) Frequencies of CMV-specific CD4⁺ T-cell PhenoGraph clusters among total CD4⁺ T-cells are shown for all measured time points. Arrows indicate the day post-transplant of CMV viremia onset. Patients are grouped according to post-transplant CMV viral load. **B)** CMV-specific CD4⁺ T-cell counts in the blood of haplo-HSCT patients experiencing CMV viremia during the first year post-transplant. Medians with the number of patients per time point are shown and error bars represent interquartile range.