

Supplementary file of manuscript entitled:

Anticipation of relapse and acute graft-versus-host disease after allogeneic peripheral blood stem cell transplantation: The fundamental role of the antigen presenting (dendritic) cells

Journal of Inflammation Research

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1. SUPPLEMENTAL METHODS:

Table S1: Collective data of the study participants , 29 HLA matched siblings and 29 recipients underwent allogeneic peripheral blood stem cell transplantations

	Donors n= 29	Recipients n= 29
Mean age ± SE [years]	20.8 ± 2.1	19.7+1.9
Sex[M/F]	20/9	14/15
Blood group[O/A/B/AB]	8 / 9 / 9 / 3	12 / 6 / 6/ 5
Virology [-ve/+ve]: -		
HCV Ab	26/3	16/13
HBs Ag	29/-	27/2
HBs Ab	19/10	18/11
HBc Ag	29/-	29/-
HBc Ab	29/-	29/-
HIV-Ab	29/-	29/-
HSV Ab	19/10	20/9
CMV[IgG]	7/22	8/21
CMV[IgM]	29/-	29/-
Myeloablative /Mini-transplantation	-	22/7

Abbreviation: M=Male; F=Female; HCV Ab=Hepatitis C virus antibody; HBs Ag= Hepatitis B surface antigen; HBs Ab=Hepatitis B surface antibody; HBc Ag=Hepatitis B core antigen; HBc Ab=Hepatitis B core antibody; HIV Ab= Human immunodeficiency virus antibodies; HSV Ab=Herpes simplex viruses antibody; CMV= cytomegalovirus; Ig=immunoglobulin

Table S2. Modified Glucksberg staging system for aGVHD

Stage	Skin (% body surface area)	Liver (bilirubin)	Gut tract (stool output/day)*
0	No GVHD rash	<2 mg/dL	Adult: <500 mL/day Child: <10 mL/kg/day
1	Maculopapular rash <25%	2-3 mg/dL	Adult: 500-999 mL/day Child: 10-19.9 mL/kg/day OR persistent nausea, vomiting, or anorexia with a positive upper GI biopsy
2	Maculopapular rash 25-50%	3.1-6 mg/dL	Adult: 1000-1500 mL/day Child: 20-30 mL/kg/day
3	Maculopapular rash >50%	6.1-15 mg/dL	Adult: >1500 mL/day Child: >30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation >5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of volume)

*For Gut staging: The "adult" stool output values are used for patients > 50kg in weight

BSA, Body surface area

Overall Clinical Grades

- Grade 0: No stage 1-4 of any organ
- Grade I: Stage 1-2 rash and no liver or GI involvement
- Grade II: Stage 3 rash and/or Stage 1 liver and/or GI involvement
- Grade III: Stage 0-3 rash with Stage 2-3 liver or GI involvement
- Grade IV: Stage 4 skin, liver, or GI involvement

Table S3. Treatment response criteria

Response	Criterion
Complete Response	Absence of any signs or symptoms of aGVHD
Partial Response	Improvement in the severity of aGVHD by at least one grade compared to Visit 1
Stable Disease	No change in the grade of aGVHD compared to Visit 1
Disease Progression	Deterioration in the severity of aGVHD by at least one grade compared to Visit 1

2. Supplemental Results

Table S4. Grafts and patients' characteristics of the study 29 recipients underwent allogeneic PBSCT.

	Sex	Underlying diagnosis	Conditioning Regimen	Kinetic assessment of the graft					aGVHD or Relapse
				TNC [X 10 ⁹ /L]	CD34+ [X10 ⁶ /kg]	CMRF -44 ⁺ DC	CD11c ⁺ DC[DC1]	CD4 ^{bright} DC[DC2]	
Group I:									
Case-1	F	SAA	Myeloablative	422	12.9	4.77	4.009	0.25	No
Case-2	M	SAA	Myeloablative	333	6.7	11.09	4.39	5.82	No
Case-3	M	SAA	Myeloablative	460	10	10.2	4.0	6.1	No
Case-4	M	MM	Non-myeloablative	540	18.8	6.9	3.1	3.5	No
Case-5	F	NHL	Myeloablative	170	3.0	4.2	2.4	2.003	No
Case-6	F	B-TM	Myeloablative	565	10.7	10.1	5.5	4.18	No
Case-7	F	B.TM	Myeloablative	272	5.8	3.2	2.16	1.25	No
Case-8	F	AML(M5)	Myeloablative	190	8.4	12.5	5.44	8.8	No
Case-9	F	AML(M1)	Non-myeloablative	900	24	7.2	6.5	0.36	No
Case-10	M	AML(M1)	Myeloablative	200	6.5	12.8	11.04	0.08	No
Case-11	F	AML(M1)	Myeloablative	370	31	9.6	4.1	3.07	No
Case-12	M	AML(M5)	Myeloablative	325	8.8	2.92	1.83	1.3	No
Case-13	M	ALL(PRO-B)	Myeloablative	370	20	11.88	2.69	3.4	No
Case-14	F	MDS(RA)	Non-myeloablative	655	9.1	13.7	5.71	11.79	No
Case-15	F	MDS	Non-myeloablative	370	25	10.5	6.65	4.7	No
Case-16	M	MDS	Non-myeloablative	565	6.6	5.1	2.43	4.3	No
Case-17	M	CML	Myeloablative	600	48	5.25	2.51	3.7	No
Case-18	F	CML	Myeloablative	400	5.8	13.4	7.8	6.04	No
Group II:									
Case-19	M	SAA	Myeloablative	324	21.5	11.72	5.63	6	aGVHD
Case-20	M	SAA	Myeloablative	980	14.0	*24.51	*19.6	5.33	aGVHD
Case-21	F	H.DL	Myeloablative	296	19.5	13.06	6.0	7.7	aGVHD
Case-22	F	SAA	Myeloablative	390	3.2	*28	*17.76	8.8	aGVHD
Case-23	F	AML(M4)	Myeloablative	500	4.3	*31.9	*21.24	10.3	aGVHD
Case-24	M	ALL(c-ALL)	Myeloablative	169	4.2	*28	*23.6	3.9	aGVHD
Case-25	M	ALL(PRO-B)	Myeloablative	450	3.0	*18.25	11.18	6.25	aGVHD
Case-26	M	ALL(T-cell)	Myeloablative	625	4.6	15.8	7.6	9.5	aGVHD
Case-27	F	AML(M1)	Non-myeloablative	400	8.2	14	8.7	4.7	aGVHD
Group III:									
Case-28	F	AML(M1)	Non-myeloablative	512	2.6	*26.6	5.9	*20.7	Relapse
Case-29	M	ALL(T-cell)	Myeloablative	540	8.9	*20	4.69	*16.14	Relapse

Abbreviations: F=Female; M=Male; TNC =Total nucleated cells; DC= dendritic cell; CMRF-44+= dendritic cell positive to CMRF-44 monoclonal antibody; SAA=Severe Aplastic Anaemia; B-TM=B-Thalassemia major; MDS=Myelodysplastic syndrome; NHL=Non Hodgkin Lymphoma; HDL=Hodgkin Lymphoma; AML=Acute Myeloid Leukaemia; ALL=Acute-lymphocytic leukaemia; CML=Chronic Myeloid Leukaemia ; MM=Multiple Myeloma; aGVHD=acute Graft-versus-host disease; PBSCT= Peripheral blood stem cell transplantation.

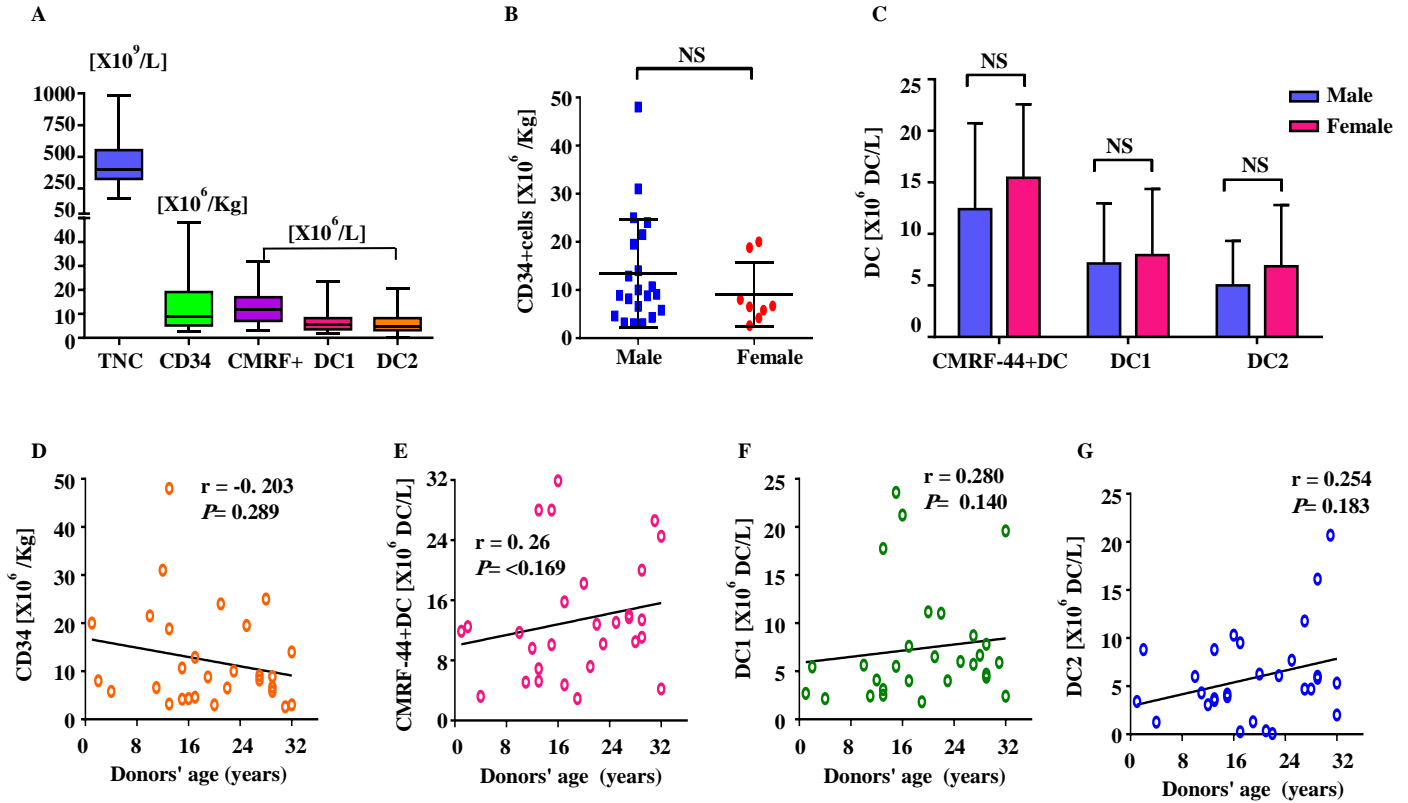


Figure S1. Graft components of the study 29 recipients underwent allogeneic PBSCT, and their relationship with donor's age and gender. (A) The bar charts represent the variable components of the grafts in 29 recipients underwent allogeneic PBSCT. (B) Comparison of CD34+ cell numbers in grafts from male and female donors. (C) Comparison of CMRF-44+DC, DC1 and DC2 cell numbers in grafts from male and female donors. Schematic box plots representing the correlations of different CD34+ cell numbers and types of DC among 29 grafts. Correlation between CD34+ cell count (D), CMRF-44+DC count (E), DC1 (F), DC2 count (F) and donors' age.

Abbreviations: TNC =Total nucleated cells; DC= dendritic cell; CMRF-44+=dendritic cell positive to CMRF-44 monoclonal antibody; r= the correlation coefficient; NS, non-significant.

Table (S5): Dendritic cell components in relation to the incidence of acute GVHD:

Dendritic cell components	Number of cases	Percentage	Grades of acute GVHD
↑ CMRF-44 ⁺ DC	One case	11.1 %	grade II
↑ CMRF-44 ⁺ DC ↑ DC1	4 cases	44.4%	-2 cases grade II -One case grade III -One case grade IV

Abbreviation: DC= dendritic cell; GVHD=acute Graft-versus-host disease.

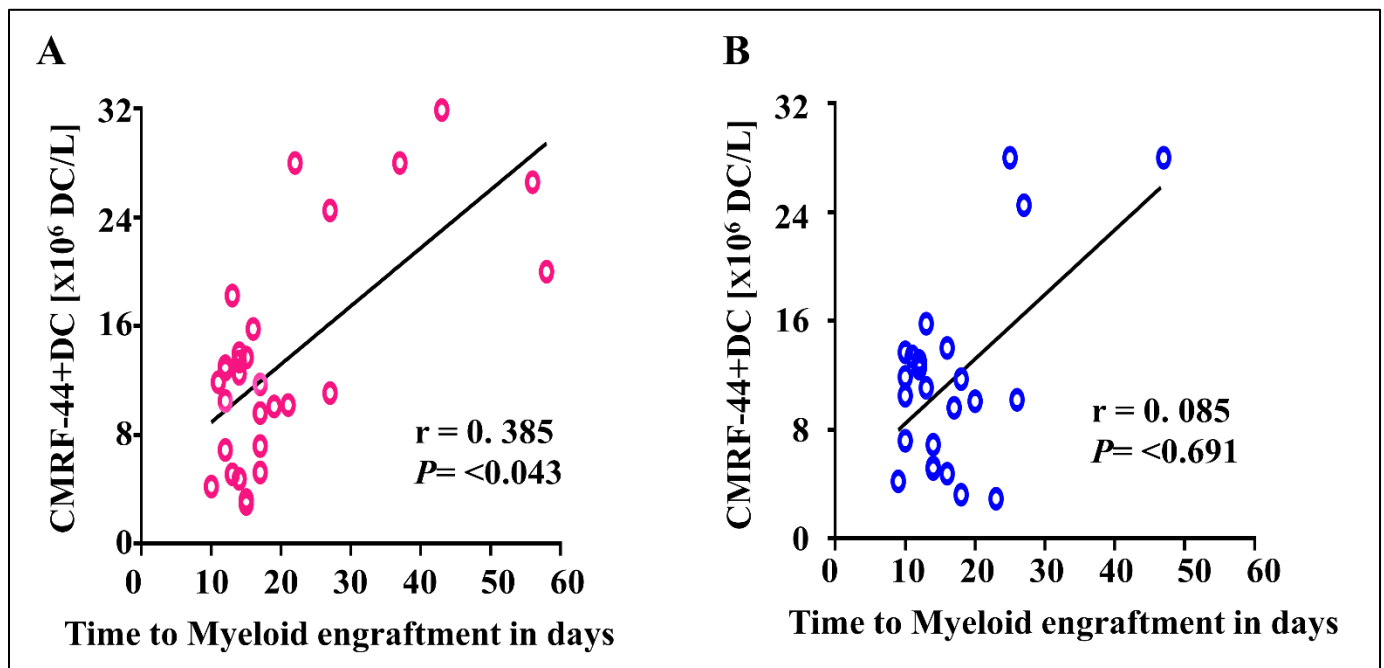


Figure S2. High DCs numbers increased days to myeloid and platelet engraftment detection in 29 recipients underwent allogeneic PBSCT. Schematic box plots representing the correlations of different time to myeloid cells (A), platelets engraftment (B) and CMRF-44+DC number in the grafts.

Abbreviations: DC= dendritic cell; CMRF-44+=dendritic cell positive to CMRF-44 monoclonal antibody; r= the correlation coefficient.

Table (S6): Multivariate Tests^c

	Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.990	1.382E2 ^a	11.000	16.000	.000	.990
	Wilks' Lambda	.010	1.382E2 ^a	11.000	16.000	.000	.990
	Hotelling's Trace	95.010	1.382E2 ^a	11.000	16.000	.000	.990
	Roy's Largest Root	95.010	1.382E2 ^a	11.000	16.000	.000	.990
Groups	Pillai's Trace	1.375	3.396	22.000	34.000	.001	.687
	Wilks' Lambda	.094	3.281 ^a	22.000	32.000	.001	.693
	Hotelling's Trace	4.630	3.157	22.000	30.000	.002	.698
	Roy's Largest Root	2.939	4.542 ^b	11.000	17.000	.003	.746
<p>a. Exact statistic</p> <p>b. The statistic is an upper bound on F that yields a lower bound on the significance level.</p> <p>c. Design: Intercept + Outcomes</p>							

Table (S7): Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Outcomes	Age (years)	275.640	2	137.820	1.819	.182	.123
	TLC (X10 ⁹ /L)	134.873	2	67.436	.262	.771	.020
	Vol (ml ³)	1265.966	2	632.983	.544	.587	.040
	TNC (X10 ⁶ /L)	31814.810	2	15907.405	.355	.705	.027
	WT (Kg)	238.471	2	119.236	.263	.771	.020
	GC (%)	192.101	2	96.050	.795	.462	.058
	CD34 ⁺ (%)	1.064	2	.532	.273	.764	.021
	CD34 ⁺ (X10 ⁶ /kg)	259.832	2	129.916	1.250	.303	.088
	DC (X10 ⁶ /L)	1070.045	2	535.022	19.479	.000	.600
	DC1 (X10 ⁶ /L)	485.597	2	242.799	12.724	.000	.495
	DC2 (X10 ⁶ /L)	424.773	2	212.386	23.672	.000	.646
Error	Age (years)	1970.222	26	75.778			
	TLC (X10 ⁹ /L)	6686.931	26	257.190			
	Vol (ml ³)	30277.000	26	1164.500			
	TNC (X10 ⁶ /L)	1165462.500	26	44825.481			
	WT (Kg)	11797.667	26	453.756			
	GC (%)	3142.649	26	120.871			
	CD34 ⁺ (%)	50.741	26	1.952			
	CD34 ⁺ (X10 ⁶ /kg)	2701.630	26	103.909			
	DC (X10 ⁶ /L)	714.142	26	27.467			
	DC1 (X10 ⁶ /L)	496.125	26	19.082			
	DC2 (X10 ⁶ /L)	233.272	26	8.972			
Total	Age (years)	12791.000	29				
	TLC (X10 ⁹ /L)	77730.631	29				
	Vol (ml ³)	1168855.000	29				
	TNC (X10 ⁶ /L)	6665679.000	29				
	WT (Kg)	102421.000	29				
	GC (%)	26730.670	29				
	CD34 ⁺ (%)	226.171	29				
	CD34 ⁺ (X10 ⁶ /kg)	7299.810	29				
	DC (X10 ⁶ /L)	6952.639	29				
	DC1 (X10 ⁶ /L)	2563.242	29				
	DC2 (X10 ⁶ /L)	1562.150	29				

Abbreviations: Preph.TLC= peripheral Total leukocytic count; Vol.= volume of the graft; TNC =Total nucleated cells; Wt=weight of the patients; GC= gated cells; DC= dendritic cell

Table (S8): Estimated Marginal Means

Dependent Variable	Groups	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Age (years)	Group I	17.667	2.052	13.449	21.884
	Group II	19.444	2.902	13.480	25.409
	Group III	30.000	6.155	17.347	42.653
TLC (X10 ⁹ /L)	Group I	48.926	3.780	41.156	56.696
	Group II	51.872	5.346	40.884	62.860
	Group III	43.240	11.340	19.930	66.550
Vol (ml3)	Group I	192.944	8.043	176.411	209.478
	Group II	207.222	11.375	183.841	230.604
	Group III	202.500	24.130	152.900	252.100
TNC (X10 ⁶ /L)	Group I	411.500	49.903	308.923	514.077
	Group II	459.333	70.573	314.268	604.399
	Group III	526.000	149.709	218.269	833.731
WT (Kg)	Group I	55.944	5.021	45.624	66.265
	Group II	53.444	7.101	38.849	68.040
	Group III	65.500	15.062	34.539	96.461
GC (%)	Group I	30.267	2.591	24.940	35.593
	Group II	24.611	3.665	17.078	32.144
	Group III	28.700	7.774	12.720	44.680
CD34+ (%)	Group I	2.494	.329	1.818	3.171
	Group II	2.523	.466	1.566	3.481
	Group III	1.750	.988	-.280	3.780
CD34+(X10 ⁶ /kg)	Group I	14.483	2.403	9.545	19.422
	Group II	9.167	3.398	2.182	16.151
	Group III	5.750	7.208	-9.066	20.566
DC (X10 ⁶ /L)	Group I	8.628	1.235	6.089	11.168
	Group II	20.582	1.747	16.991	24.173
	Group III	23.300	3.706	15.682	30.918
DC1 (X10 ⁶ /L)	Group I	4.570	1.030	2.454	6.686
	Group II	13.479	1.456	10.486	16.472
	Group III	5.295	3.089	-1.054	11.644
DC2 (X10 ⁶ /L)	Group I	3.480	.706	2.029	4.931
	Group II	6.942	.998	4.890	8.995
	Group III	18.400	2.118	14.046	22.754

Abbreviations: Preph.TLC= peripheral total leukocytic count; Vol.= volume of the graft; TNC =total nucleated cells; Wt=weight of the patients; GC= gated cells; DC= dendritic cell