

Supplemental methods

Table 1. List of clones and providers of Abs used for FACS analysis in the mouse experiments.

Antigen	Clone	Fluorochrome	Manufacturer
CD3	145-2C11	PC5	BD bioscience
CD4	GK1.5	BV786	BD Biosciences
CD8a	53-6.7	APC-H7	BD Biosciences
CD8a	53-6.7	BV711	BD Biosciences
CD25	PC61,5	PE-Cy7	eBiosciences
CD39	24DMS1	alexa 700	Thermo Fisher
CD45	30-F11	APC-H7	BD Biosciences
CD62L	mel-14	PE	Miltenyi
CD103	"2E7"	FITC	Miltenyi
CD107A	1D4B	PE-Cy TM 7	BD Biosciences
CTLA-4	UC10-4F10-11	PE-CF594	BD Biosciences
H-2Kd	SF-1.1	PE	eBioscience
ICOS	REA192	APC-H7	Miltenyi
KLRG1	2F1	PE-Cy TM 7	Thermo Fisher
Ly-6C	AL-21	BV605	BD bioscience
Nrp-1	3DS304M	PC5	Thermo Fisher
TNFR-1	55R-286	PE	Biolegend
TNFR2	REA228	APC	Miltenyi
TNFR2	REA228	PE	Miltenyi
TNFR2	TR75-89	BV421	BD Biosciences

INTRACELLULAR

Antigen	Clone	Fluorochrome	Manufacturer
Foxp3	FJK-16S	eF450	eBiosciences
Ki-67	B56	BV650	BD Biosciences
KI-67	B56	AL488	BD Biosciences
Granzyme B	NGZB	FITC	Thermo Fisher
IFNg	B27	BV711	BD Biosciences

FIXABLE VIABILITY STAIN (FVS)

Antigen	Clone	Fluorochrome	Manufacturer
FVS		AlexaFluor R700	BD Biosciences
FVS		BV505	BD Biosciences

Table 2. List of clones and providers of Abs used for FACS analysis of human cells.

Antigen	Clone	Fluorochrome	Manufacturer
CD3		V500	BD Biosciences
CD4	RPA-T4	APC-H7	BD Biosciences
CD45RA	HI100	BB™ 515	BD Biosciences
CD25	M-A251	PE-Cy™7	BD Biosciences
CD25	2A3	PE-Cy™7	BD Biosciences
TNFR2	Rea	PE	Miltenyi
CTLA-4	BN13	PE-CF594	BD Biosciences
PD-1	EH12-1	BV™ 605	BD Biosciences

INTRACELLULAR

Antigen	Clone	Fluorochrome	Manufacturer
FoxP3	PCH101	eF450	eBiosciences
FoxP3	259D	PE	BioLegend
Ki-67	B56	BV™ 650	BD Biosciences
HELIOS		APC	eBiosciences

FIXABLE VIABILITY STAIN (FVS)

Antigen	Clone	Fluorochrome	Manufacturer
FVS		AlexaFluor R700	BD Biosciences
FVS		V510	Thermo Fisher

Supplemental table S1. Patient characteristics and clinical outcome

Variables	GVHD Patients (N=7)	Relapse Patients (N=6)
Gender, N (%)		
Recipients – M/F	5/2 (71/29)	1/5 (16.7/83.3)
Donors – M/F	3/4 (43/57)	3/3 (50/50)
Age (years), median (range)		
Recipients	53 (18-64)	48.5 (29-72)
Donors	35 (0-58)	34 (22-45)
HLA matching, N (%)		
Matched related donor	2 (29)	1 (16.7)
Matched unrelated donor	2 (29)	3 (50)
Mismatched unrelated donor	3 (42)	1 (16.7)
Haplo-identical donor		1 (16.7)
ABO matching, N (%)		
ABO compatibility	4 (57)	3 (50)
Minor incompatibility	1 (14)	2 (33.3)
Major incompatibility	2 (29)	1 (16.7)
Graft source, N (%)		
PBSC	6 (86)	6 (100)
CBU	1 (14)	
Hematologic malignancies, N (%)		
Acute leukemia	3 (42)	5 (83.3)
Chronic myeloid leukemia	1 (14)	
Myeloproliferative disorder	1 (14)	1 (16.7)
Myelodysplastic syndrome	2 (29)	
Conditioning regimen, N (%)		
Myeloablative	1 (14)	2 (33.3)
Reduced intensity	6 (86)	2 (33.3)
Sequential		2 (33.3)
GVHD prophylaxis, N (%)		
CsA/ATG	1 (14)	1 (16.7)
CsA/MMF +/- ATG	5 (72)	3 (50)
CsA/MTX		1 (16.7)
CsA	1 (14)	
PTCy/CsA/MMF		1 (16.7)
Acute GVHD (maximum grading), N (%)		
Grade II	1 (14)	
Grade III - IV	6 (86)	
Acute GVHD treatment, N (%)		
corticosteroids	7 (100)	
etanercept	4 (57)	
ruxolitinib	3 (42)	
Alpha-1 antitrypsin	2 (29)	
vedolizumab	1 (14)	
Time from transplant to relapse (months), median (range)		9.5 (0-21)
Time from transplant to last follow-up (months), median (range)		19 (6-38)
Clinical outcome, N (%)		
Corticosteroid resistant	7 (100)	
Complete response after 2 nd line or more	1 (14)	
Partial response after 2 nd line or more	1 (14)	
Deceased (GVHD-related)	5 (72)	
Alive		4 (66.7)
Deceased		2 (33.3)

Abbreviations:

N: number; M: male; F: female; PBSC: peripheral blood stem cell; CBU: Cord Blood Unit; ATG: anti-thymocyte globulin; GvHD: Graft-versus-Host Disease; aGvHD: acute GvHD; CsA: cyclosporine A; MMF: mycophenolate mofetil, MTX: methotrexate, PTCy: post-transplant cyclophosphamide.