SUPPLEMENTARY APPENDIX

A phase I study of CD25/regulatory T-cell-depleted donor lymphocyte infusion for relapse after allogeneic stem cell transplantation

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Supplemental Methods.

A. Flow Cytometry Reagents and Cell Populations Analyzed within CD25/Treg-depleted DLI Product

Antibody-Fluorochrome	**Clone**	**Supplier**	**Target**
Anti-CD45-PerCP	2D1	BD Bioscience	Extracellular
Anti-CD4-FITC	RPA-T4	BD Bioscience	Extracellular
Anti-CD25-PE	M-A251	BD Bioscience	Extracellular
Anti-CD127-APC-Alexa 750	eBioRDR5	EBIOSCIENCE	Extracellular
Anti-FoxPE-Alexa 647	259D/C7	BD Bioscience	Intracellular
Cell Populations	**Expression Profile**	**Alternate**	**Alternate**
Tregs	CD45+CD4+ CD25+FoxP3+	CD45+CD4+ CD25high	CD45+CD4+ CD25med-high CD127lo

B. Flow Cytometry Reagents and Cell Populations Analyzed within Recipient Peripheral Blood Lymphocytes

Antibody-Fluorochrome	**Clone**	**Supplier**	**Target**
Anti-CD3-V450	UCHT1	BD Bioscience	Extracellular
Anti-CD4-APC-H7	RPA-T4	BD Bioscience	Extracellular
Anti-CD8-Pacific Orange	3B5	Invitrogen	Extracellular
Anti-CD25-PE-Cy7	M-A251	BD Biosciences	Extracellular
Anti-CD127-PE-Cy5	eBioRDR5	EBIOSCIENCE	Extracellular

Anti-CD56-PE	B129	BD Bioscience	Extracellular
Anti-CD20-APC-H7	2H7	BD Bioscience	Extracellular
Anti-CD45RO-FITC	UCHL1	BD Bioscience	Extracellular
Anti-CD62L-APC	Dreg 56	BD Bioscience	Extracellular
Anti-CD11c-PE-Cy5	B-ly6	BD Bioscience	Extracellular
Anti-CD123-FITC	AC125	Miltenyi Biotec	Extracellular
Cell Population	**Expression Profile**	**Cell Population**	**Expression Profile**
Tregs	CD3+CD4+ CD25med-high CD127low	Tcons	CD3+CD4+ CD25neg-low CD127med-high
Natural Killer Cells	CD3-CD56+	B Cells	CD20+
Dendritic Cells	Lineage - HLA-DR+		
Naive T Cells	CD45RO-CD62L+	Central Memory T Cells	CD45RO+CD62L+
Terminal Effector T Cells	CD45RO-CD62L-	Effector Memory T Cells	CD45RO+CD62L-

Intracellular surface markers and intracellular FoxP3 expression was detected using the FACSCanto II system (BD Biosciences, San Jose, CA), and FACSDiva software (BD Biosciences).

C. Additional Statistical Definitions

OS was defined as time from DLI to death. EFS was defined as time from DLI to relapse, disease progression, further treatment, or death, whichever occurred first.

Supplemental Table 1. Detailed Demographics and Outcomes of Subjects Receiving CD25/Treg-depleted DLI

No.	Sex	Age at DLI (yrs)	Initial Dz	Donor	Prior Active GvHD	Mos from relapse to DLI	Mos from HSCT to DLI	Type of Txs/ Last dosing prior to DLI	Dz Status at DLI	% Marrow Blasts	Donor Leukocyte Chimerism at DLI	Disease Status at 8-12 weeks	Status at 1 year	Disease Status at 1 year	Type of GvHD	Time from DLI to Death (mos)	Cause of Death
Dose Level 1 - 1x10^7 CD3+/kg																	
1	M	68.9	2o AML	MRD	None	0.6	10.4		Untx	6%	89%	SD	D			2.7	Relapse
2	M	29.2	1° AML	MRD	Grade I aGvHD	1.3	5.5		Untx	40%	32%	Prog	D			2.4	Relapse
3	F	42.6	2° AML	MRD	None	2.3	6.2	7+3 6 wks	Prog	20%	69%	Prog	D			1.4	Relapse
4	M	37.8	ALL	MRD	None	1.7	5.8	HiDACx1 6 wks	PR	1% (EM)	100%	Prog	D		Extensive cGvHD	3.2	Relapse
5	M	31.4	HL	MRD	Extensive cGvHD	5.3	21.4	GNDx4 5 wks	Prog	N/A	99%	Prog	A	Persistent – on Txs	Limited cGvHD	8.6	Relapse
6	M	22.7	HL	MUD	Extensive cGvHD	7.7	28.5	Panobinostat x15 6 wks	Prog	N/A	100%	Prog	A	Persistent – on Txs			
Dose Level 2 - 3x10^7 CD3+/kg																	
7	M	55.8	1° AML	MUD	Limited cGvHD	4.7	16.6	MECx1 >4 mos	PR	5%	99%	CR	A	CR	Limited cGvHD		
8	F	19.8	1° AML	MUD	None	1.6	8.1	HiDAC x1 6 wks	PR	10%	100%	CR	A	CR	Grade I aGvHD, Extensive cGvHD		
9	F	61.6	2° AML	MRD	None	2.8	24.6	MECx1 10 wks	CR	<3%	100%	CR	A	CR		34.8	Relapse
10	F	46.8	1° AML	MUD	None	1.4	11.0	XRT only 6 days	PR	2% (EM)	100%	CR	A	CR			
11	F	41.9	1° AML	MRD	None	1.3	7.3		Untx	5-10%	93%	PR	D		Extensive cGvHD	4.1	Relapse
12	M	71.7	2° AML	MUD	None	14.7	17.5	HMAx3 4 wks	Prog	21%	45%	Prog	D			2.5	Relapse
13	M	36.7	1° AML	MUD	Grade I aGvHD	4.2	20.5	MECx1; Clofarabinex1 4 wks	PR	5%	97%	Prog	D			3.0	Relapse
14	F	63.8	MDS-> AML at DLI	MUD	None	1.9	5.1		Untx	25%	46%	Prog	D			2.5	Relapse
15	M	46.6	ALL	MRD	Extensive cGvHD	3.8	38.0	HyperCVAD >3 mos	CR	2%	99%	CR	A	Relapsed		11.7	Relapse
16	M	23.5	ALL	MRD	None	5.4	12.9	HyperCVAD 4 weeks	Prog	4% (EM)	100%	Prog	D			0.6	Relapse
17	M	45.3	ALL	MRD	None	5.5	8.3	Clofarabine; Asaparginase 7 wks	CR	<5%	100%	Prog	D			0.9	Relapse
18	M	48.7	HL	MRD	None	2.9	9.5		Untx	N/A	98%	CR	A	Relapsed	Extensive cGvHD		
19	M	25.0	HL	MRD	None	2.7	6.4	XRT only 3 days post	PR	N/A	82%	CR	A	Relapsed			

20	F	63.1	NHL	MUD	None	1.9	7.0	Asparaginase/ Dex; ICEx1 4wks	PR	N/A	83%	CR	D		Grade III ->V aGvHD	1.1	GvHD
21	F	33.4	NHL	MRD	None	6.4	36.8	BRx5 >4 wks	CR	N/A	100%	Prog	A	CR after further Txs	Grade I aGvHD		

MRD – matched related donor; MUD – matched unrelated donor; Prog – progressive disease; Untx – untreated; Dz – disease; Txs – treatments
Chemotherapy regimens: 7+3 - idarubicin and cytarabine; HiDAC – high dose cytarbine; GND – gemcitabine, vinorelbine, doxorubicin; MEC – mitoxantrone, etoposide, cytarabine; XRT – Xray radiation therapy; HMA – hypomethylating agent; Dex – dexamethasone; ICE – ifosfamide, carboplatin, etoposide; BR – bentamustine rituxumab ; EM – extramedullary disease; N/A – not applicable; SD – stable disease; NR – not reached; A – Alive; D – Deceased

Supplemental Table S2. Demographics of Subjects Receiving CD25/Treg-depleted and Contemporaneous Cohort Receiving Unmanipulated DLI

Characteristics	Treg-depleted DLI	Unmanipulated DLI	
Subjects Evaluable	n=15	n=14	p-value
Male Gender	8 (53.3%)	9 (64.3%)	0.71
Median Age, years (range)	46 (19-71)	54 (22-70)	0.24
Malignancy			0.78
AML/MDS	8 (53.3%)	8 (57.1%)	
ALL	3 (20.0%)	1 (7.1%)	
Hodgkin Lymphoma	2 (13.3%)	2 (14.3%)	
NHL/CLL	2 (13.3%)	2 (14.3%)	
Myeloma	0	1 (7.1%)	
Allogeneic HSCT			
Donor Source			1
HLA-matched Sibling Donor	8 (53.3%)	8 (57.1%)	
HLA-matched Unrelated Donor	7 (46.7%)	6 (42.9%)	
Conditioning Intensity			0.46
Myeloablative	8 (53.3%)	5 (35.7%)	
Reduced-Intensity	7 (46.7%)	9 (64.3%)	
Prior Acute GvHD			1
Grades I	1 (6.7%)	1 (7.1%)	
Grades II-IV	0	0	
Prior Chronic GvHD			
Limited	1 (6.7%)	0	1
Extensive	1 (6.7%)	1 (7.1%)	
Median Time HSCT to Relapse, months (range)	6.6 (2.7-30.2)	8.3 (2.4-26.1)	0.93
Median Time Relapse to DLI, months(range)	2.9 (1.6-14.7)	3.4 (0.9-6.3)	0.56
Median Time HSCT to DLI, months (range)	10.4 (5.1-38)	11.3 (4.1-27.3)	0.66
Received Cytoreductive Therapy prior to DLI	12 (80.0%)	9 (64.2%)	0.68
Median # Therapies (range)	1 (1-2)	1 (1-3)	
Disease Status Prior to DLI			0.31
Complete Remission	4 (26.7%)	5 (35.6%)	
Partial Response	6 (40.0%)	2 (14.3%)	
Untreated	3 (20.0%)	4 (28.6%)	
Progressive Disease after Cytoreduction	2 (13.3%)	3 (21.4%)	
Median Total Donor Chimerism (range)	99% (46-100)	97% (22-99)	0.14
Median Cell Dose of DLI $x10^7$/kg (range)	3.0	3.0 (2.2-3.7)	

Supplemental Table S3. Detailed Demographics and Outcomes of Comparator Subjects Receiving Unmanipulated DLI

No.	Sex	Age at DLI (yrs)	Initial Dz	Donor	Prior Active GvHD	Mos from relapse to DLI	Mos from HSCT to DLI	Type of Txs/ Last dosing prior to DLI	Dz Status at DLI	% Marrow Blasts	Donor Leukocyte Chimerism at DLI	DLI CD3+ Cell Dose (10^7/kg)	Disease Status at 8-12 weeks	Status at 1 year	Disease Status at 1 year	Time from DLI to Death (mos)	Cause of Death
1	F	39.5	1^{o} AML	MUD	None	4.6	10.1	MECx1 3 mos	CR	N/D (EM)	99%	2.3	CR	D		5.2	Relapse
2	M	60.4	1^{o} AML	MRD	None	1.2	27.2	XRT only 4 wks	CR	1%	99%	3.5	CR	D		11.3	Relapse
3	F	45.8	1^{o} AML	MRD	None	4.0	24.2	HiDACx1 8 wks	CR	1%	97%	3.0	Prog	A	Relapsed – on Txs	14.3	Relapse
4	M	70.6	2^{o} AML	MUD	None	1.4	4.1		Untx	25%	57%	3.0	Prog	A	Relapsed – on Txs	16.2	Relapse
5	F	64.0	1^{o} AML	MRD	None	1.2	6.7		Untx	16%	22%	3.0	Prog	D		2.3	Relapse
6	F	48.7	1^{o} AML	MRD	None	3.5	6.9	MECx1 Sorafenib 1 wk	PR	50%	75%	3.0	Prog	D		1.2	Relapse
7	M	63.4	1^{o} AML	MUD	None	4.4	14.5	HiDAC x1 ME HMA x1 2 wks	Prog	40%	43%	3.0	Prog	D		1.2	Relapse
8	M	52.3	MDS	MRD	Grade 1 aGvHD	3.4	23.9		Untx	5%	99%	3.7	Prog	A	Relapsed – on Txs	15.1	Relapse
9	M	49.9	ALL	MRD	None	2.76	7.30	MECx1 POMPx1 3wks	CR	3%	99%	3.0	Prog	D		4.8	Relapse
10	M	57.0	NHL	MRD	None	3.85	6.22	Gemx3 2 wks	PR	N/A	98%	3.6	SD	D		9.0	Relapse
11	M	57.4	CLL	MRD	None	0.89	8.95		Untx	N/A	80%	3.0	Prog	D		1.6	Relapse
12	M	22.7	HL	MUD	Extensive cGvHD	6.28	17.14	XRT GNDx4 >3 wks	Prog	N/A	99%	3.0	Prog	A	Relapsed – on Txs	21.9	Relapse
13	F	39.8	HL	MUD	None	3.06	14.84	GNDx2 4 wks	CR	N/A	99%	3.0	Prog	A	Relapsed – on Txs		
14	M	58.2	MM	MUD	None	3.88	12.40	RDx2 4wks	Prog	N/A	97%	2.2	Prog	D		3.6	Relapse

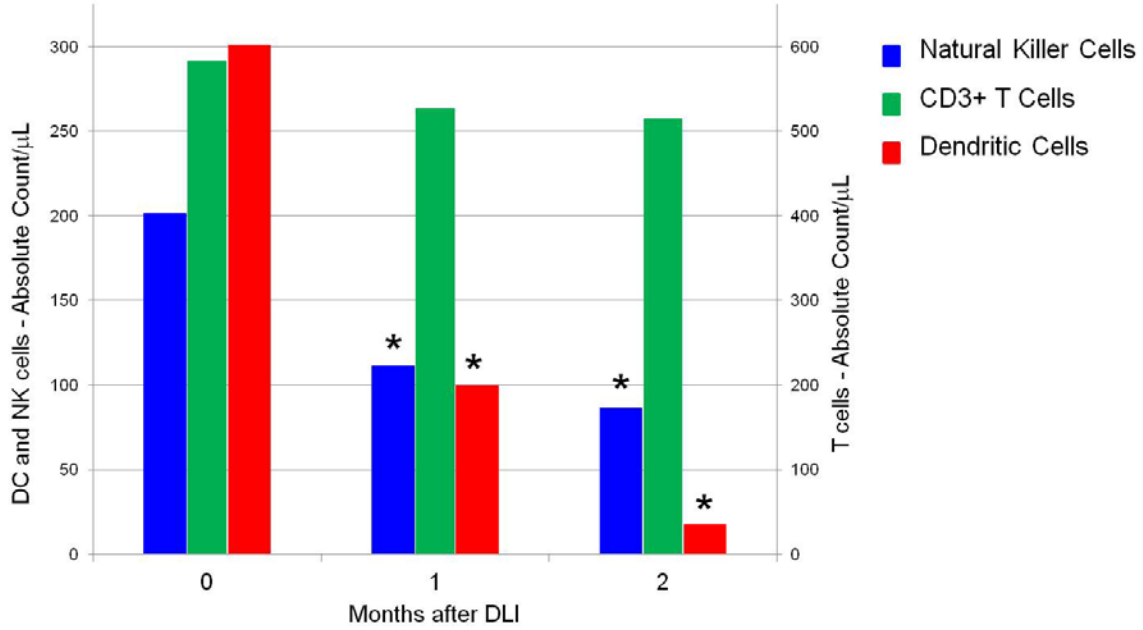
ME – mitoxantrone, etoposide; POMP – 6-mercaptopurine, vincristine, methotrexate, prednisone; Gem – Gemcitabine; RD – lenalidomide dexamethasone; N/D - not done

N.B. There was no GvHD noted following DLI infusion in this comparator cohort

Supplemental Figure S1. Numbers of natural killer and dendritic cells but not total T, Tcon, or Treg cells change after CD25/Treg-Depleted DLI. Peripheral blood mononuclear cells drawn at indicated times were stained for extracellular markers and analyzed by flow cytometry. A. NK, T-cell, and DC numbers: Median number of cells/μL in entire study group for CD3-CD56+ NK cells (blue bars, left vertical axis), Lineage-HLA-DR+ DCs (red bars, left vertical axis), and CD3+ T cells (green bars, right vertical axis), are displayed either prior to (0 months) or in the first 2 months after DLI. * indicates $p<0.05$ for comparison between baseline and the indicated time point. B. Treg numbers and Treg:Tcon ratios: Median number of Treg cells/ μL in peripheral blood (left panel) and Treg:Tcon ratios (right panel) in subjects who did (blue bars) and did not respond (red bars) to CD25/Treg-depleted DLI are displayed. P values for all comparisons to baseline were not significant.

Supplemental Figure S1. Numbers of natural killer and dendritic cells but not total T, Tcon, or Treg cells change after CD25/Treg-Depleted DLI.

A. NK, T-cell, and DC numbers



B. Treg numbers and Treg:Tcon ratios