Allogeneic HCT without GVHD

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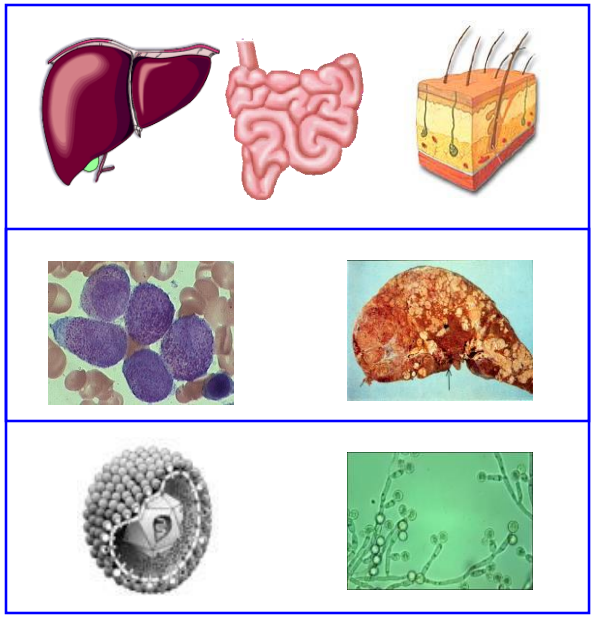
Allogeneic HCT

T cells

HLA typing

Immunosuppressive therapy



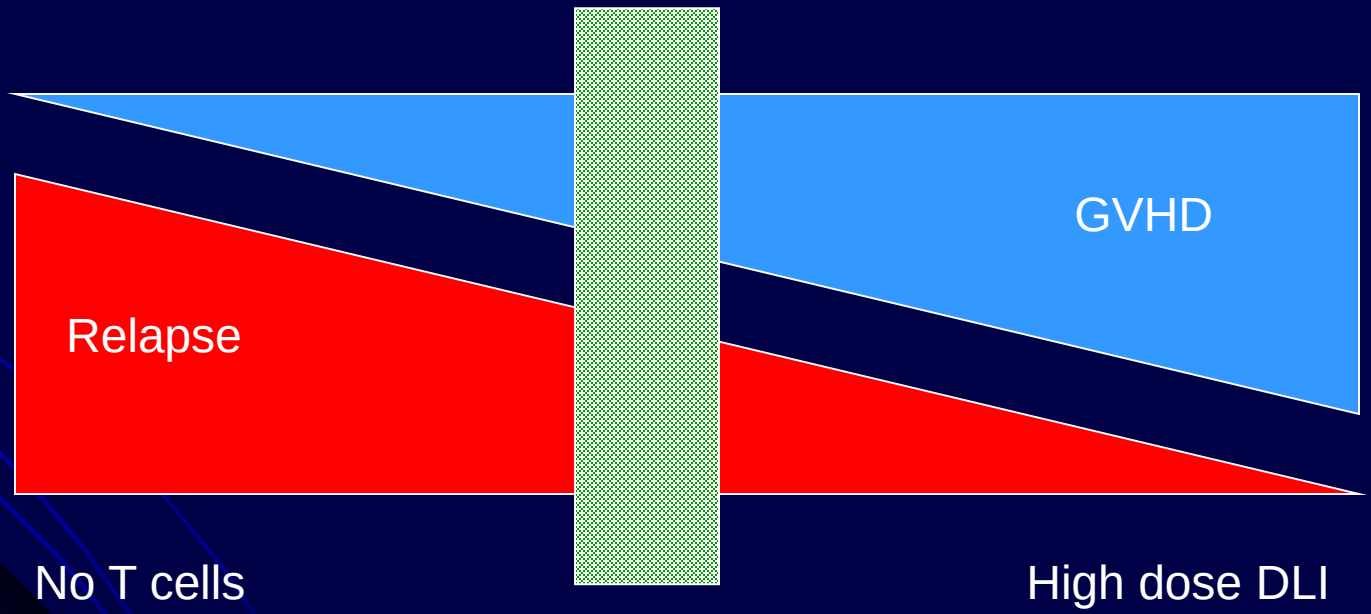
? GVHD

? GVL or GVT

? Anti-microbial

Cause of GVHD…
No T cell = No GVHD

GVHD vs. GVL



Holy Grail

How to transfer of allogeneic T-cell immunity without causing GVHD

Effector memory T cells

- **$CD62L^-$**
- **Do not cause GVHD**
- **Contribute to T cell reconstitution directly and by enhancing *de novo* T-cell regeneration from T-cell depleted bone marrow**
- **Deplete host radioresistant T cells**

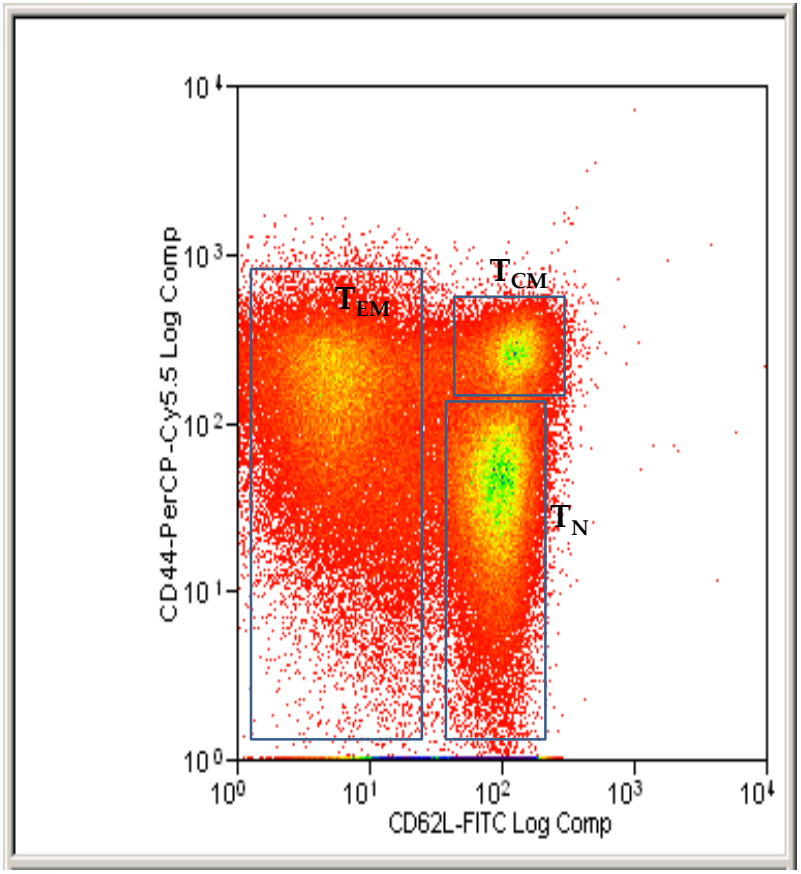
(Chen BJ et al. Blood, 2004,103:1534)

Questions: 1. Functional
2. Mechanism

T Cell Subsets

Naïve T cells (T_N)

CD62L+CD44^{low}



Central Memory T cells (T_{CM})

CD62L+CD44^{high}

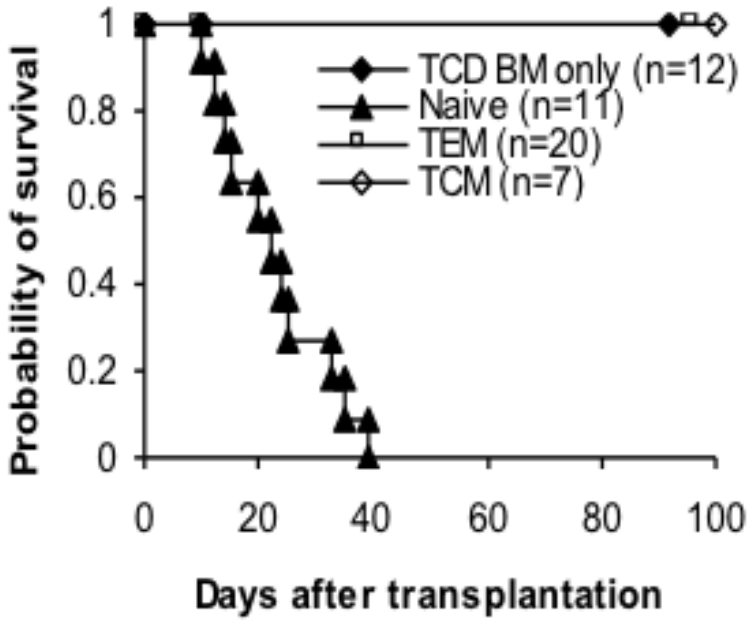
Effector Memory T cells (T_{EM})

CD62L-CD44^{high}

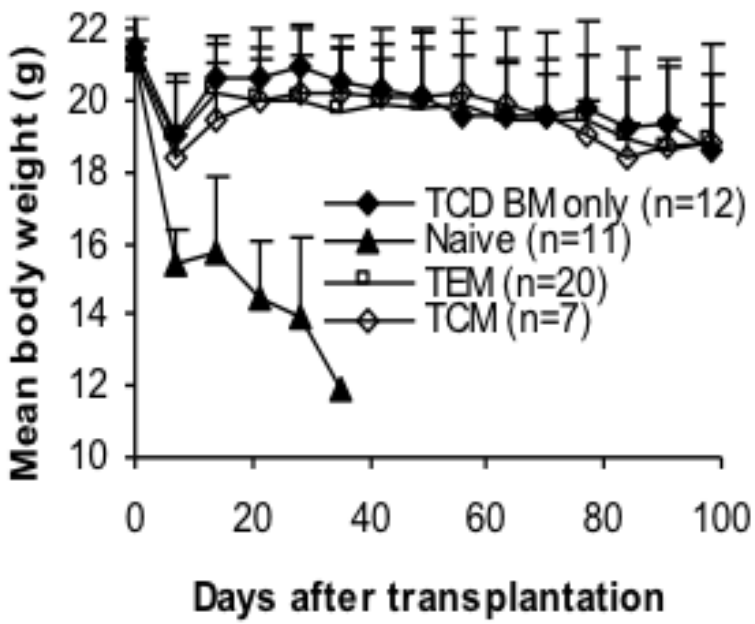
GVHD: TEM vs. TCM

Unprimed C57BL/6 ($H2^b$)→BALB/c ($H2^d$)

A. Survival



B. Weight curve



Chen BJ et al. Blood,2007,109:3115

CD62L^{-} T cells promote functional immune recovery against tumor

BCL1 model

Donor: TCD BM $1x10^7$

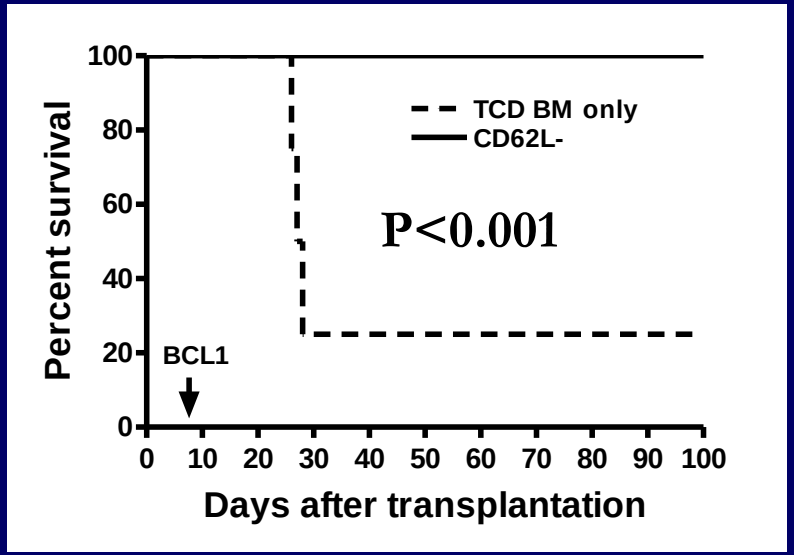
CD62L^{-} T cells $1x10^6$

Recipient: BALB/c 8.5 Gy

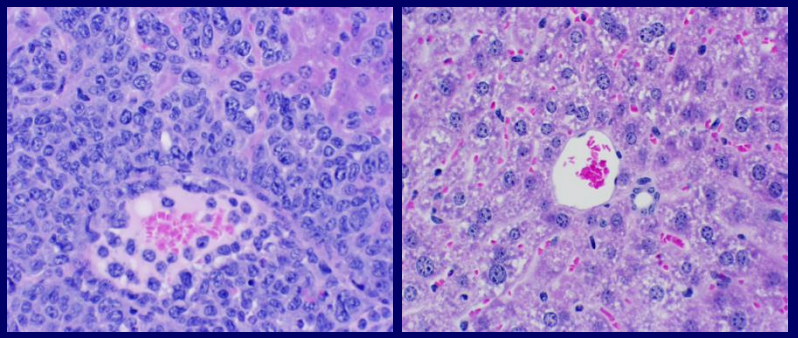
- BCL1 cells: $5x10^5$, i.p., on day +7

(a kind gift from Drs. Defu Zeng and Samuel Strober)

A. Survival



B. Histology



TCD BM only CD62L^{-}

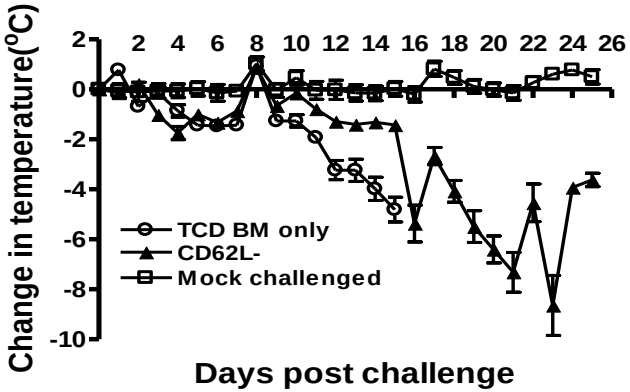
CD62L$^-$ T cells promote functional immune recovery against influenza virus

Flu model

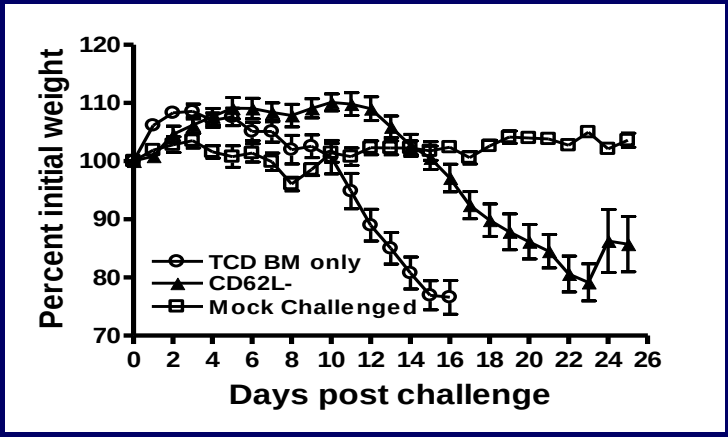
Donor:	TCD BM	$1x10^7$
	CD62L$^-$ T cells	$1x10^6$
Recipient:	BALB/c	8.5 Gy

- Influenza virus: $1.4x10^2$ pfu, i.n., day +7

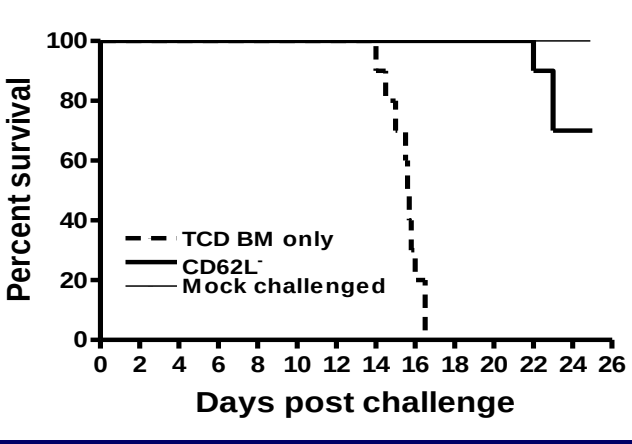
A. Temperature



B. Body Weight



C. Survival

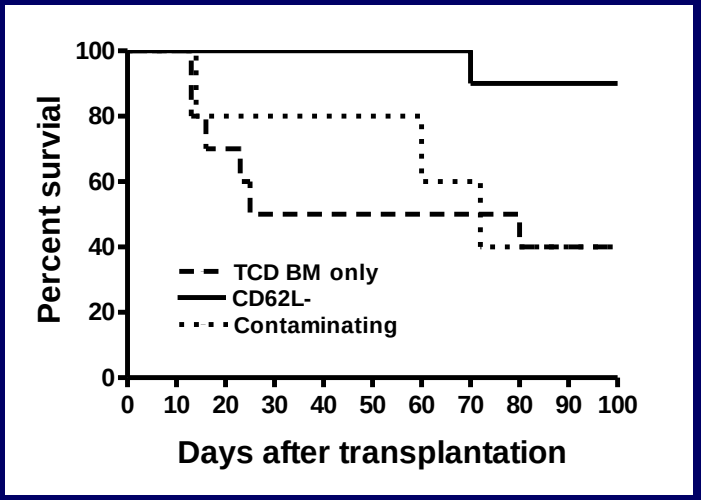


CD62L^{-} T cells facilitate hematopoietic engraftment

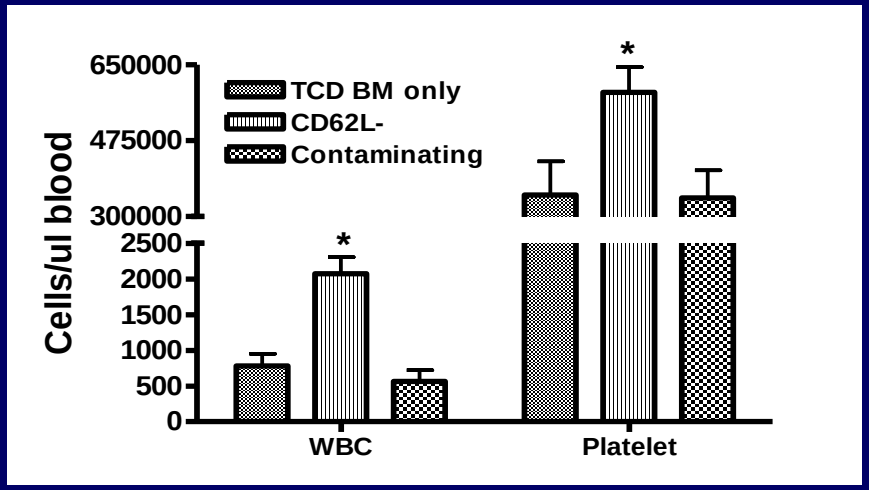
Model

Donor:	**TCD BM**	**$5x10^5$**
	CD62L^{-} T cells	**$1x10^6$**
Recipient:	**BALB/c**	**8.5 Gy**

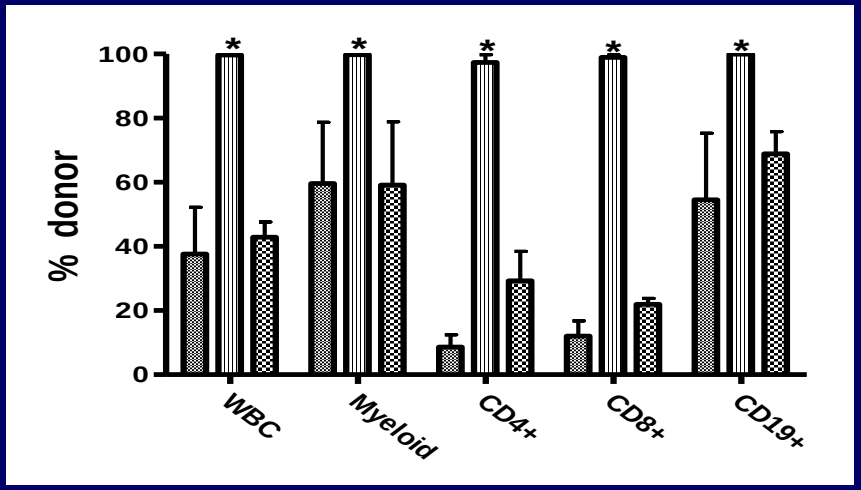
A. Survial



B. WBC and platelet

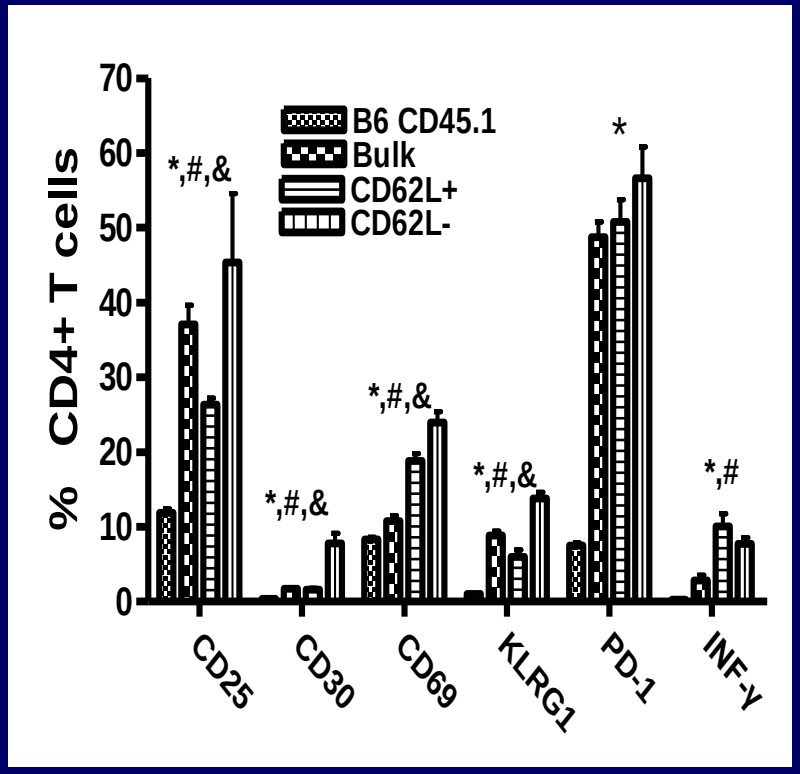


C. Chimerism

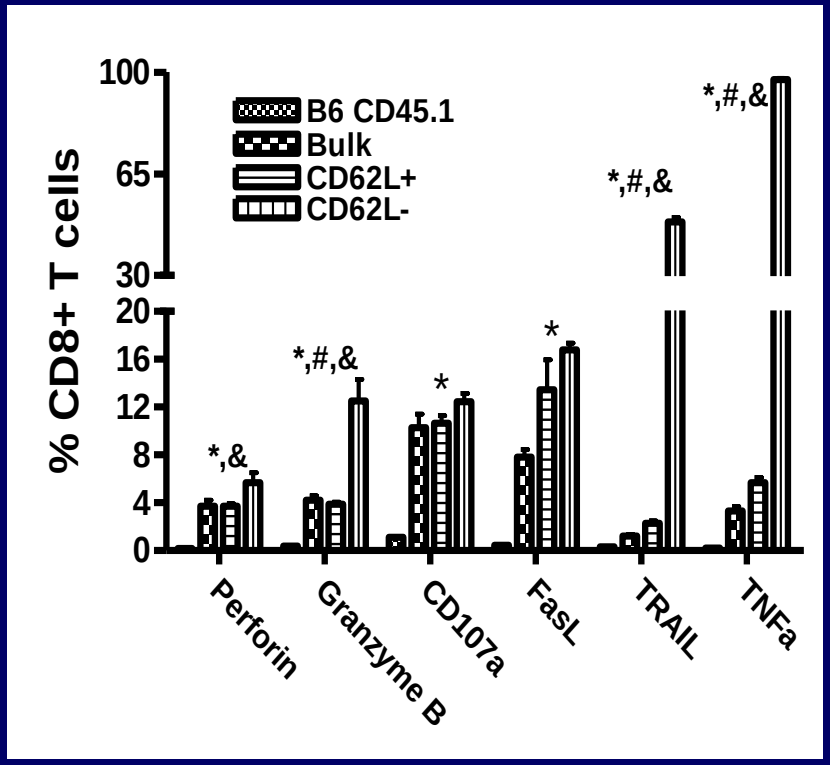


CD62L^{-} T cells are activated and express cytotoxic molecules and cytokines upon transfer into allogeneic recipients

A. CD4



B. CD8



At day 6 after transplantation

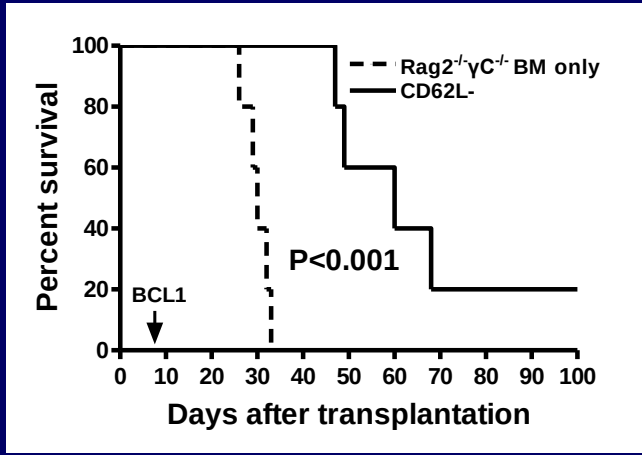
CD62L^{-} T cells lose alloreactivity over time upon transfer into allogeneic recipients

Model

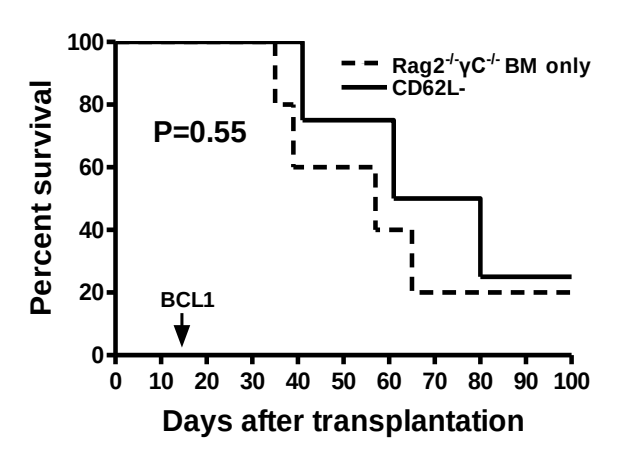
Donor:	Rag2$^{-/-}\gamma C^{-/-}$ BMC	1x10^{7}
	CD62L^{-} T cells	1x10^{6}
Recipient:	BALB/c	8.5 Gy

- BCL1 cells: 5x10^{5}, i.p.

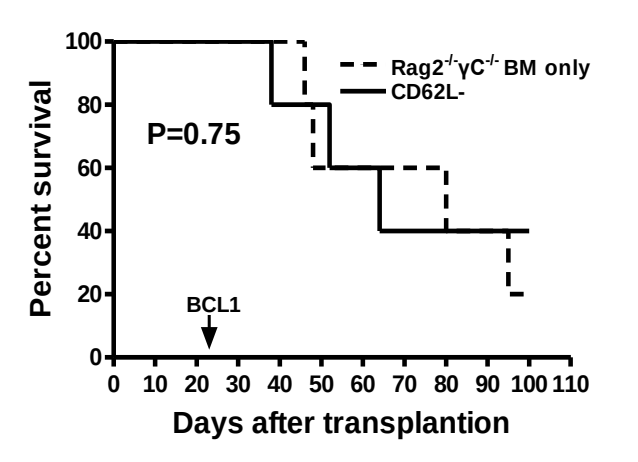
A. Day +7



B. Day +14

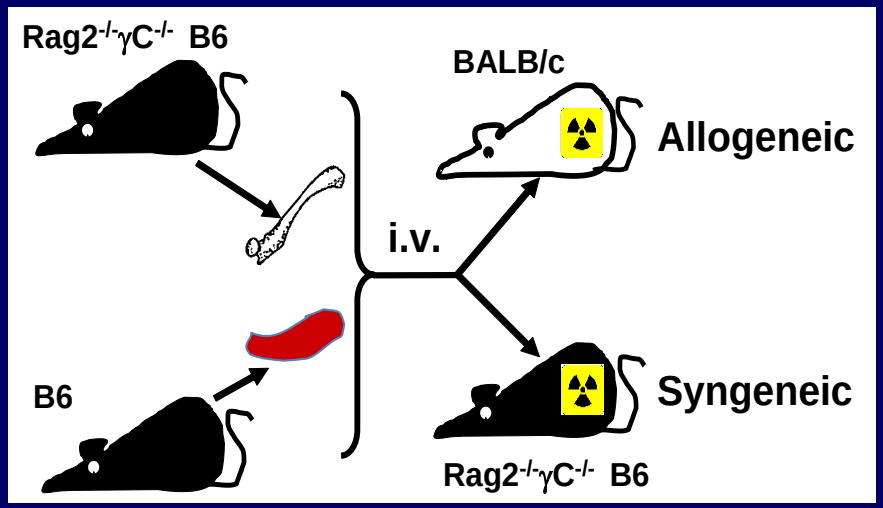


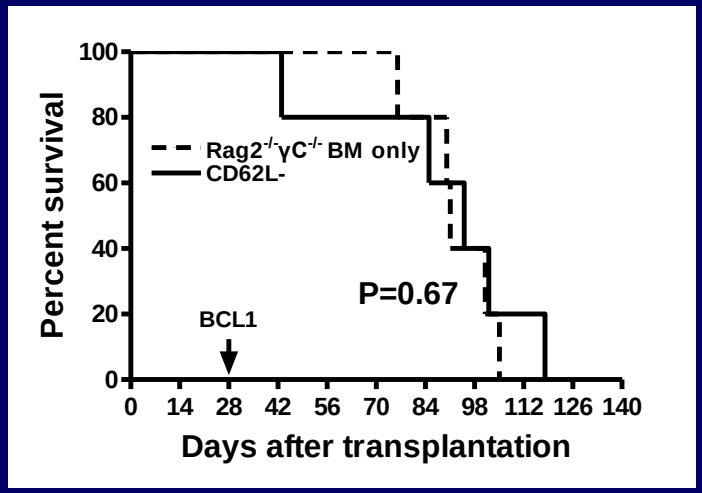
C. Day +21

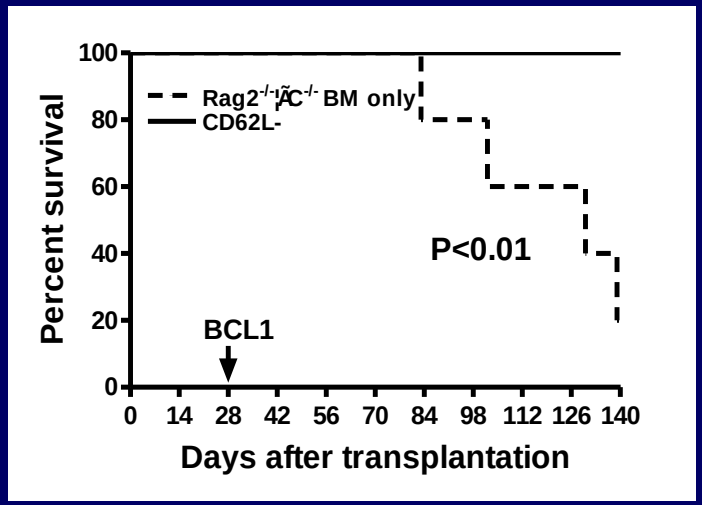


CD62L^{-} T cells lose alloreactivity over time upon transfer into allogeneic but not syngeneic recipients

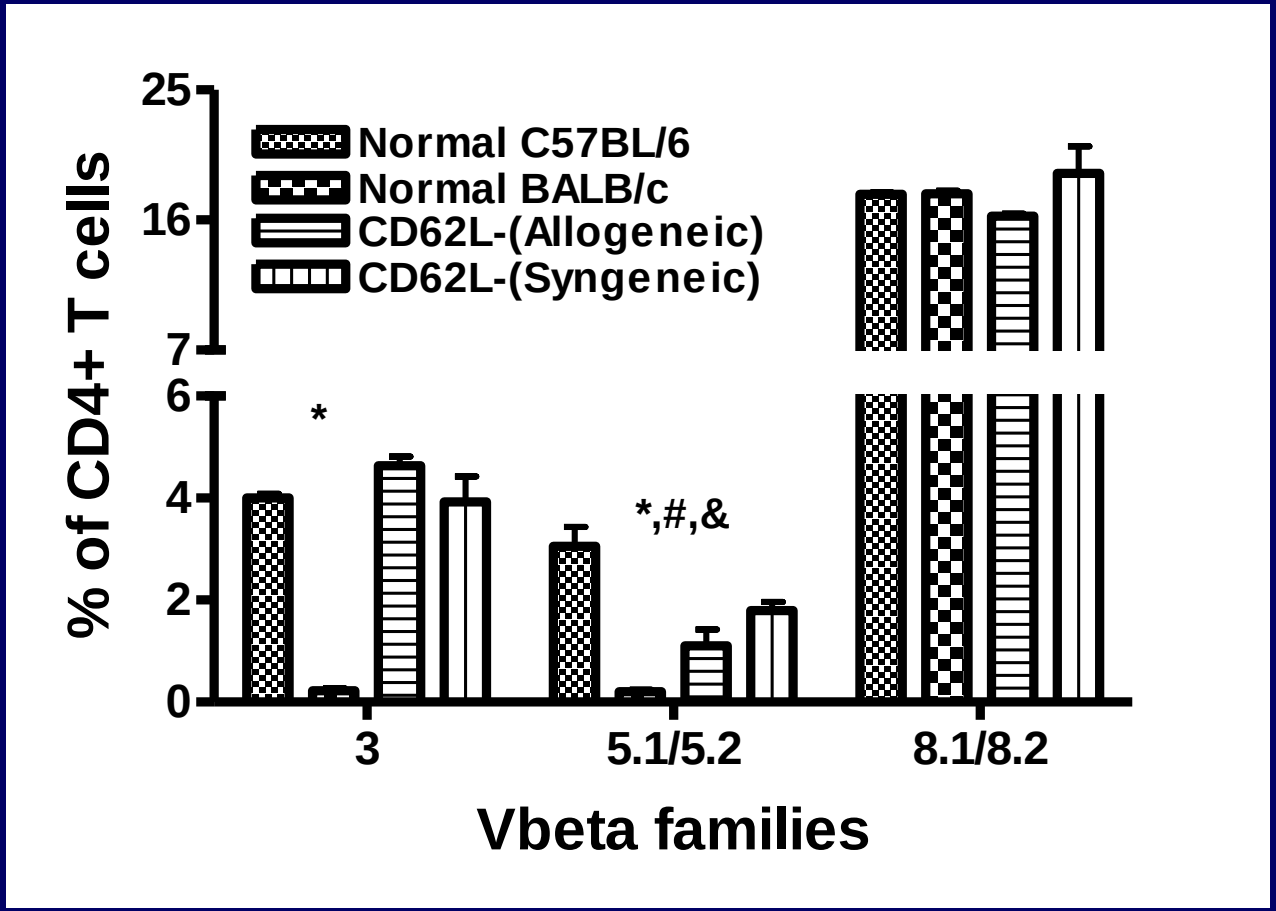
Hypothesis: Gradual loss of CD62L^{-} T cells' ability to respond to alloantigens is a result of prolonged exposure to alloantigens.







CD62L^{-} T cells may become anergic and there is partial clonal deletion in allogeneic recipients

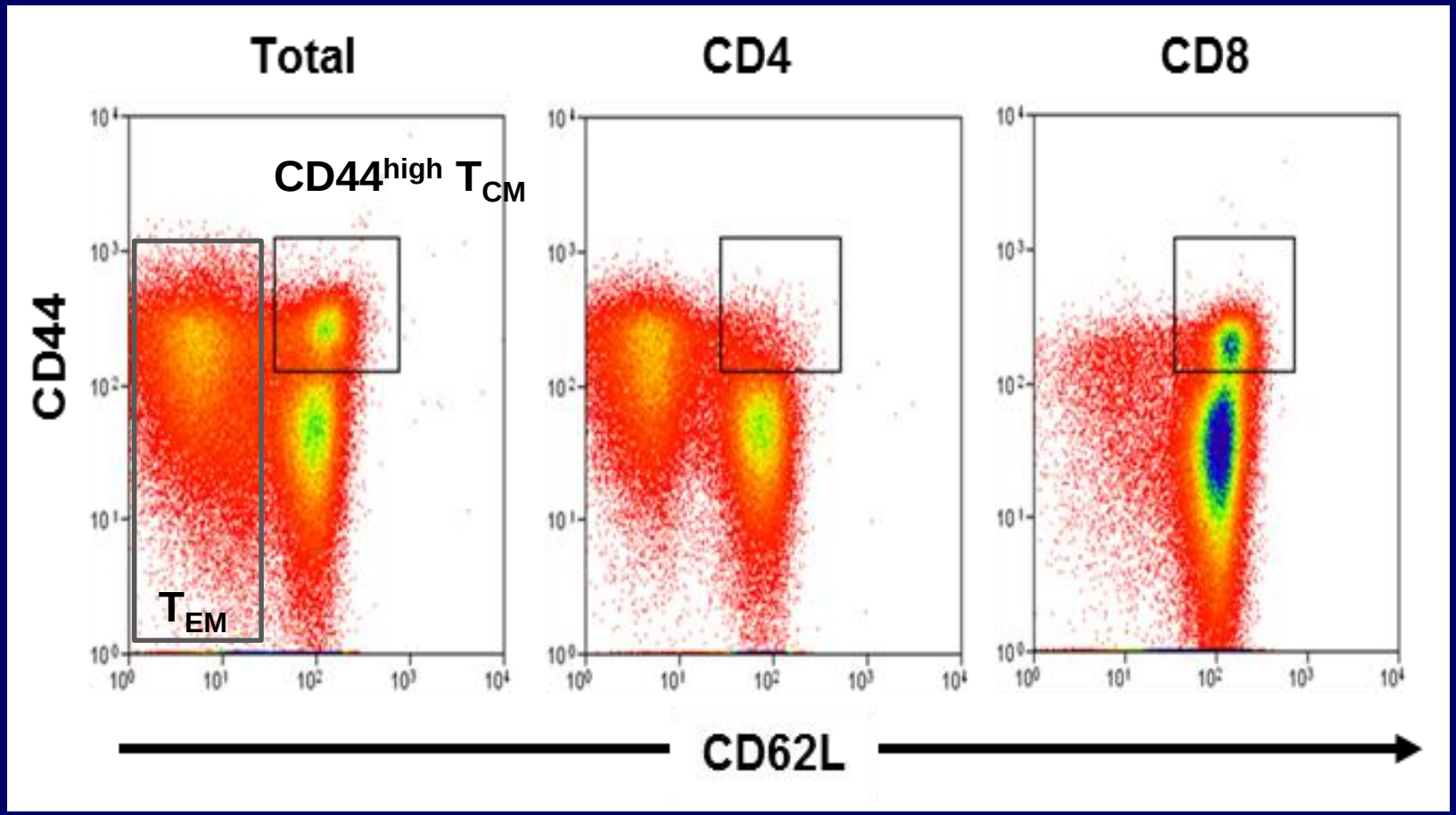


Similar results were obtained in CD8 T cells

Conclusion I

- T_{EM} do not induce GVHD, have GVL effect, enhance engraftment and have anti microbial effects.
- The role of T_{CM} in GVHD is somewhat controversial.

$CD44^{high}$ T_{CM}



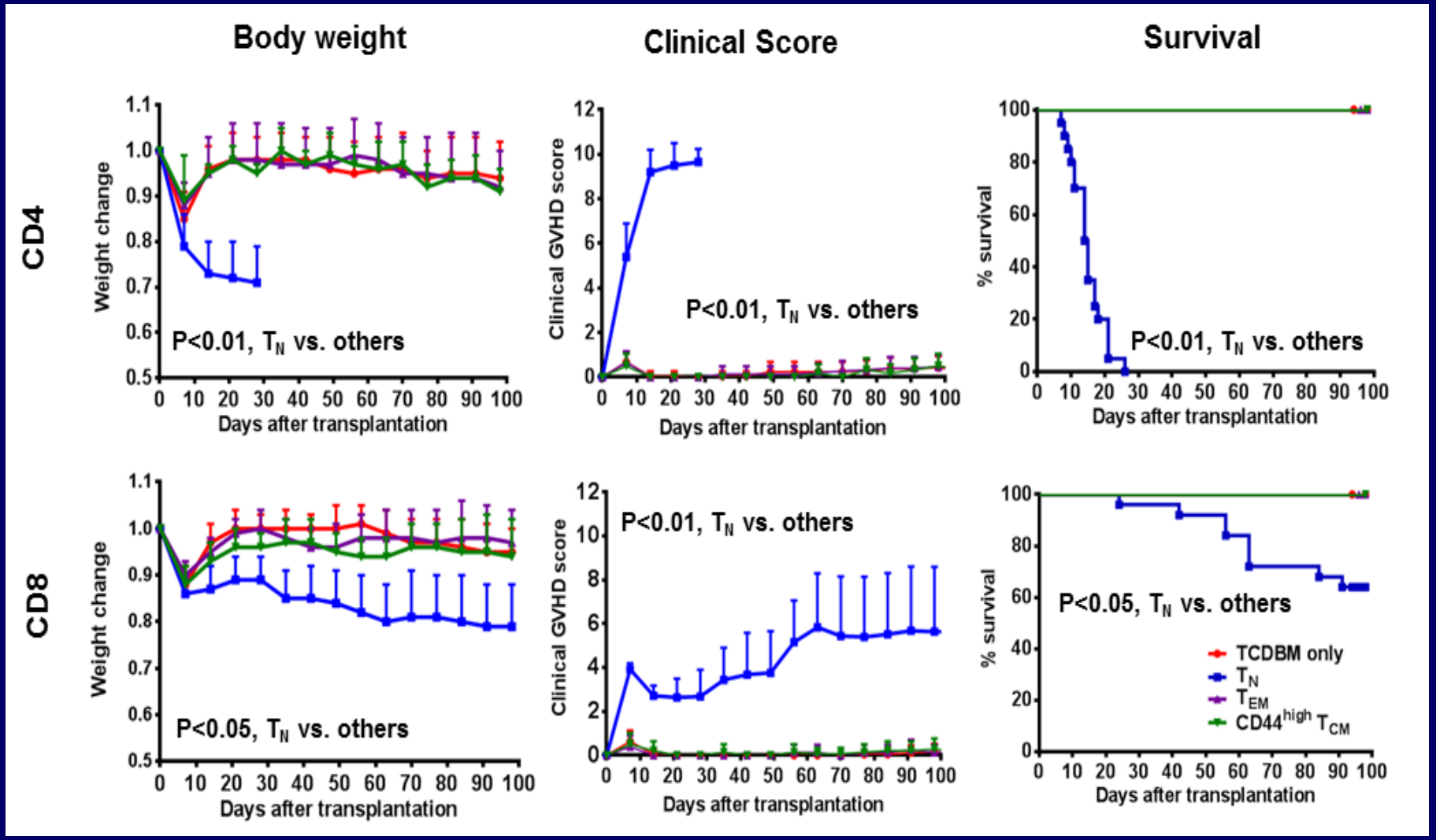
T_N: $CD62L^{+}CD45RB^{+}CD44^{-/low}$
T_{EM}: $CD62L^{-}$

% $CD44^{high}$ T_{CM}

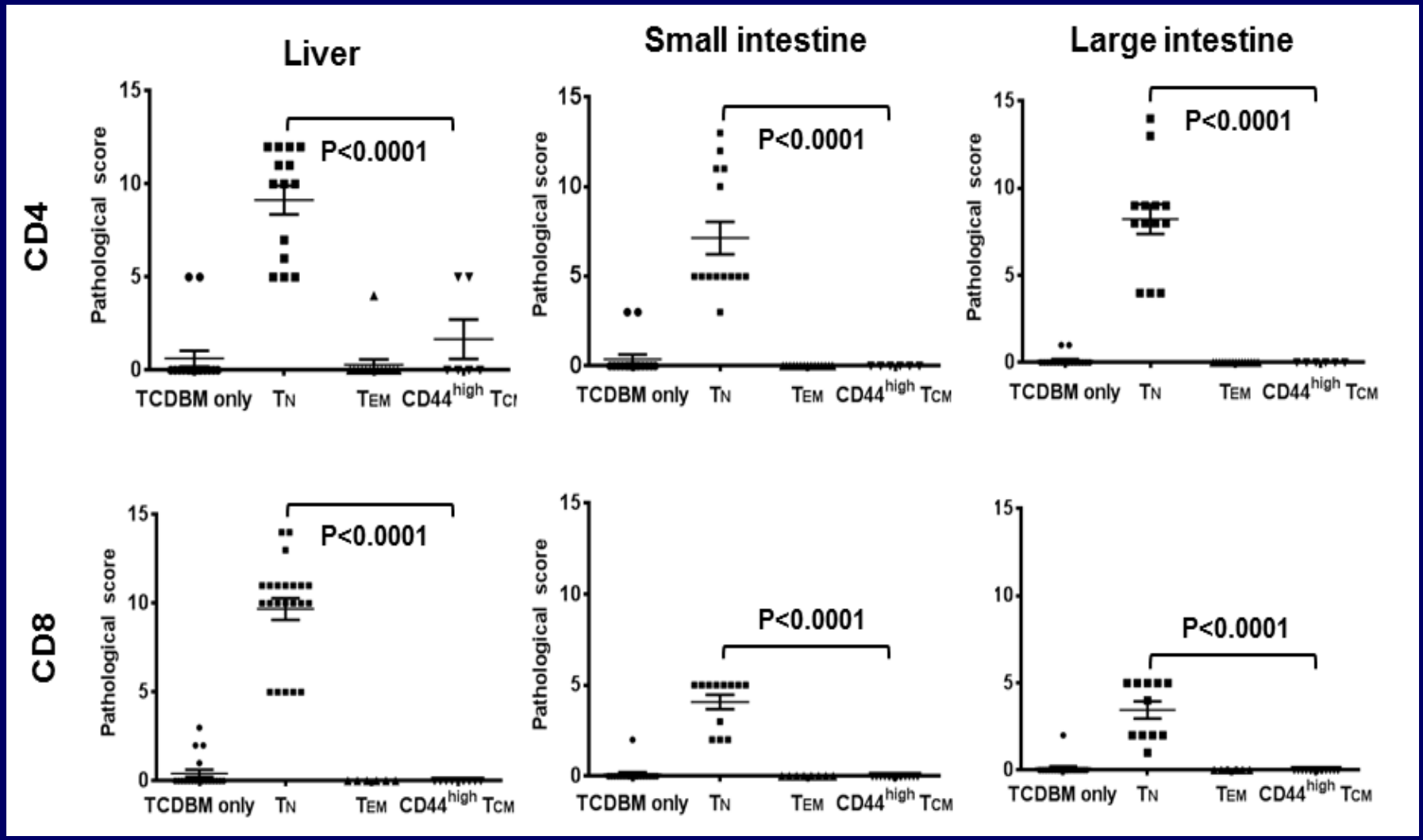
Age	Total			CD4			CD8		
	T_N	T_{EM}	$CD44^{high}$ T_{CM}	T_N	T_{EM}	$CD44^{high}$ T_{CM}	T_N	T_{EM}	$CD44^{high}$ T_{CM}
3 months	58±3	26±2	16±0	67±2	24±1	7±1	66±1	6±1	27±1
7 months	37±4*	46±5*	15±2	46±4*	45±5*	6±1	61±3*	9±1*	29±2
15 months	7±3*	71±9*	20±6	10±3*	79±5*	10±2*	13±4*	35±10*	49±8*

*P<0.05, compared with 3 months

CD44^{high} T_{CM} Do Not Induce GVHD

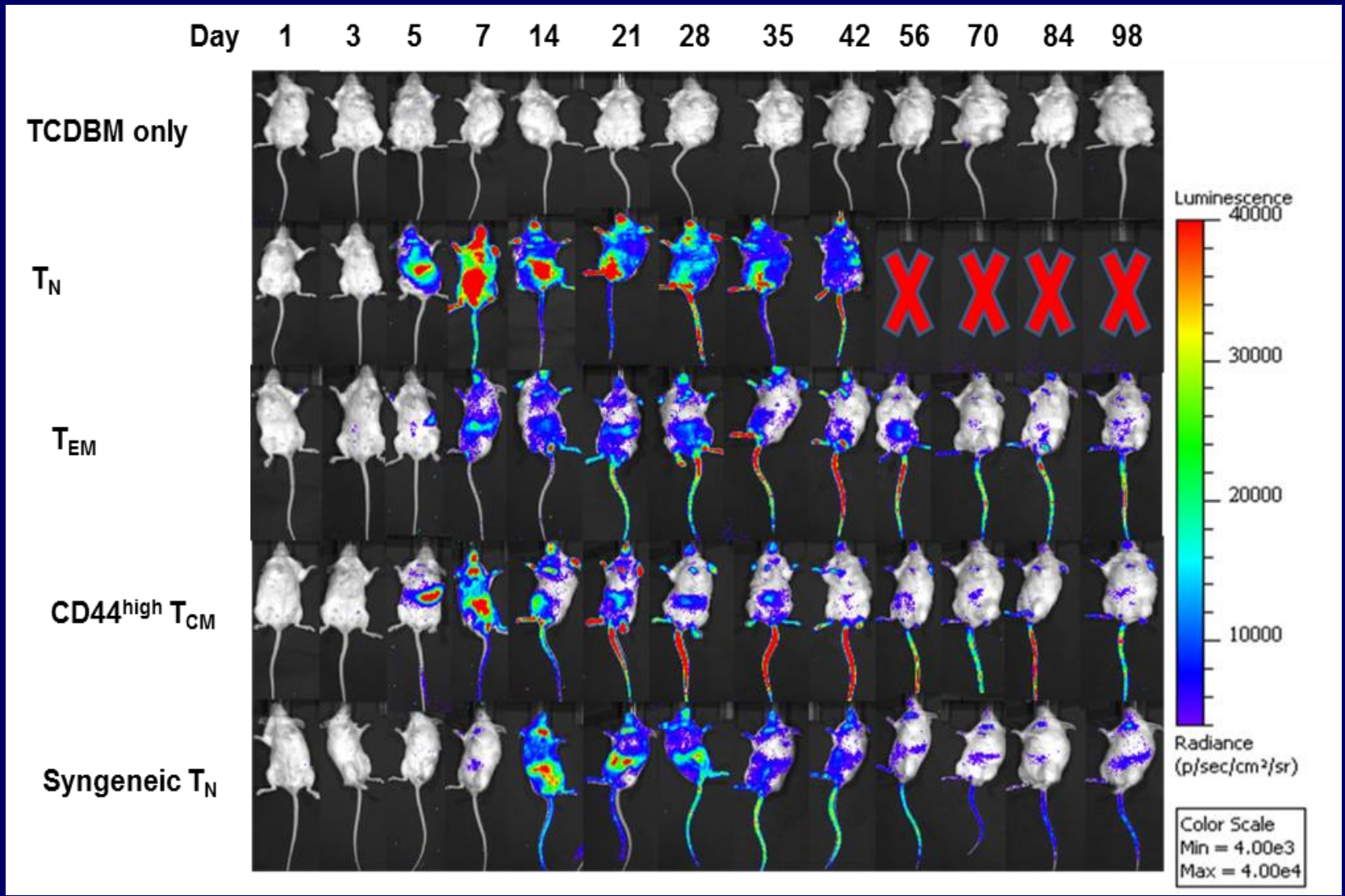


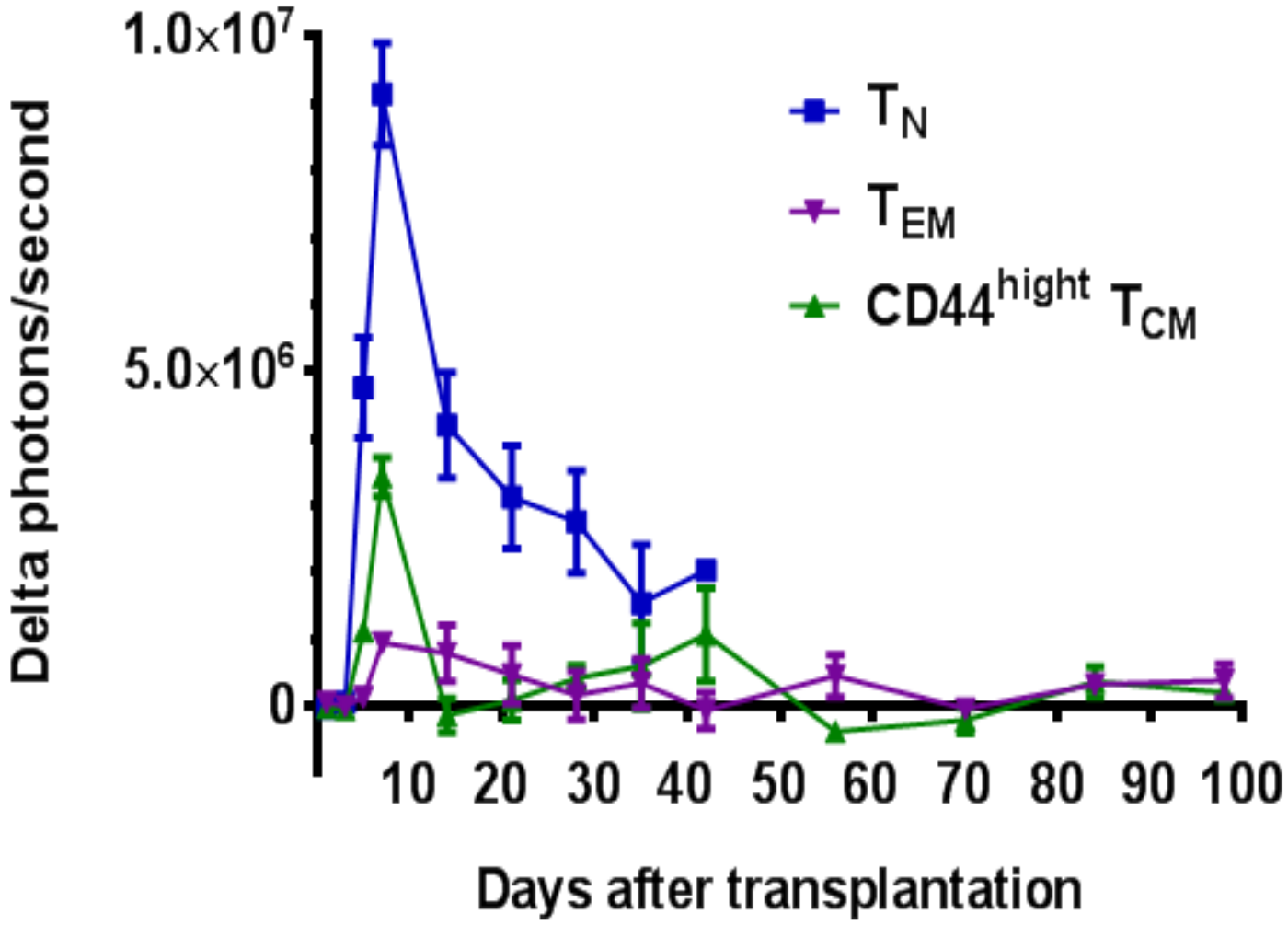
Pathological Score



Chen Liu, University of Florida

In Vivo T Cell Expansion




1.0×10^7
5.0×10^6
0
Delta photons/second
10 20 30 40 50 60 70 80 90 100
Days after transplantation
T_N
T_EM
CD44^hight T_CM

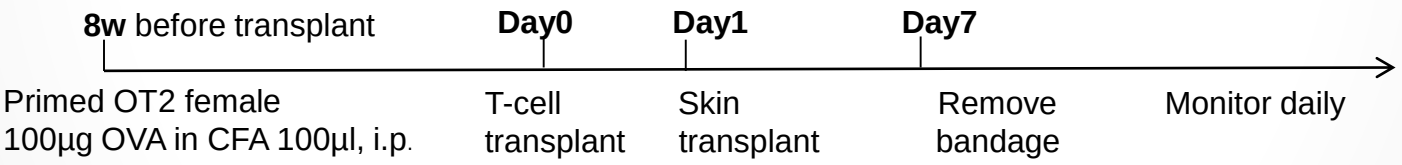
Determine T_M Memory Function

- Memory T-cell function verification:
  - Secondary skin graft rejection

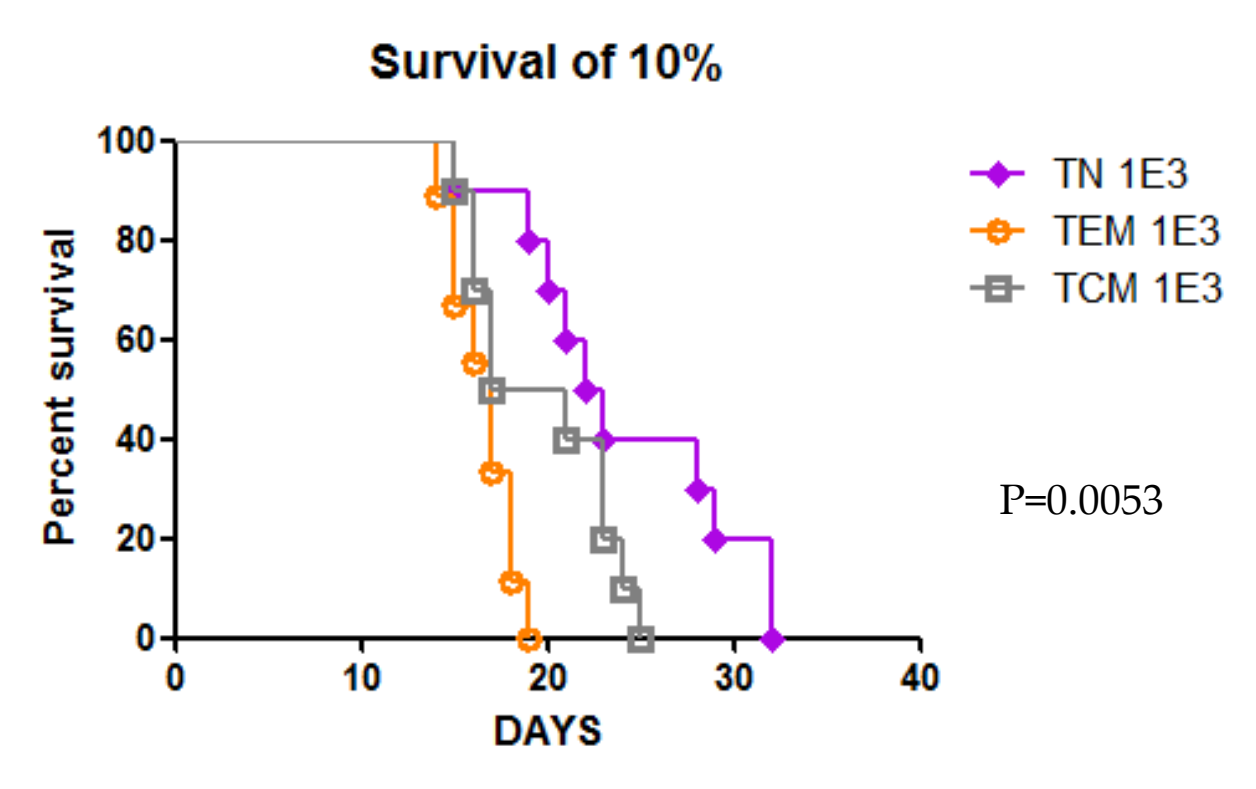
OVA skin → B6

Primed OT2 T_M → B6

Group	RPMI	Primed T_N	Primed T_{EM}	Primed T_{CM}
cell dose	/	1E3	1E3	1E3



Result: T_{EM} and T_{CM} reject skin faster than T_N



CD44^{high} T_{CM} and T_N Are Equally Potent in Facilitating Stem Cell Engraftment

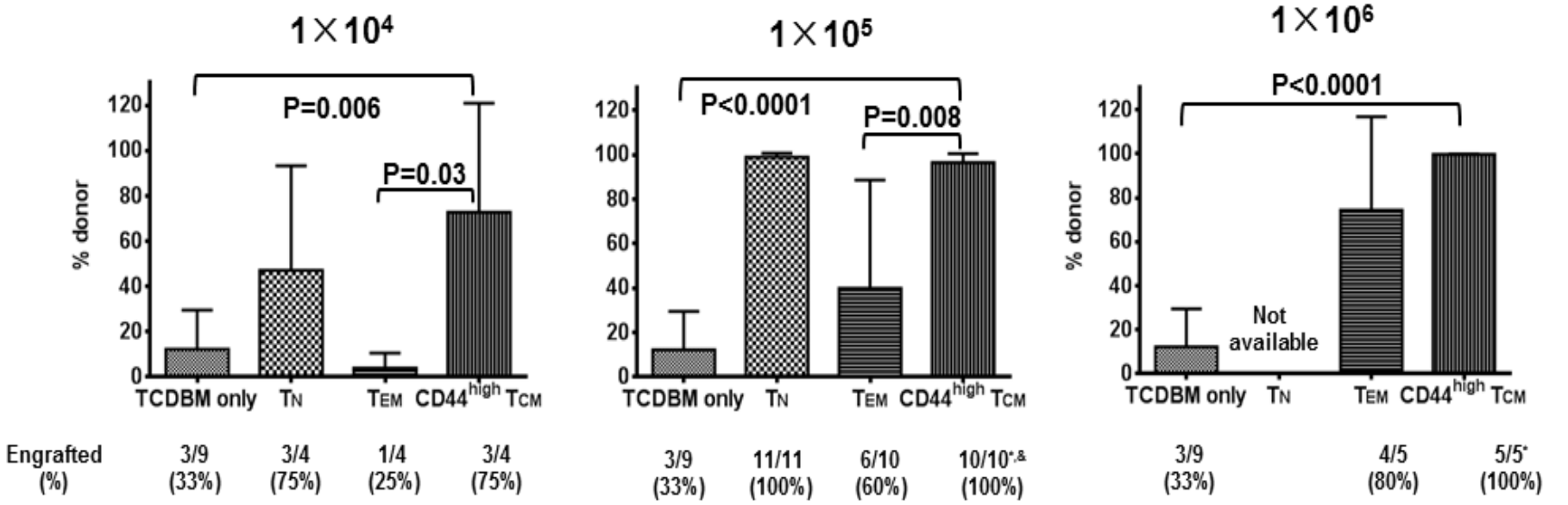
Model

Graft: **B6.SJL TCD BMC 1x10^6**

C57BL/6 T cells 3 different doses

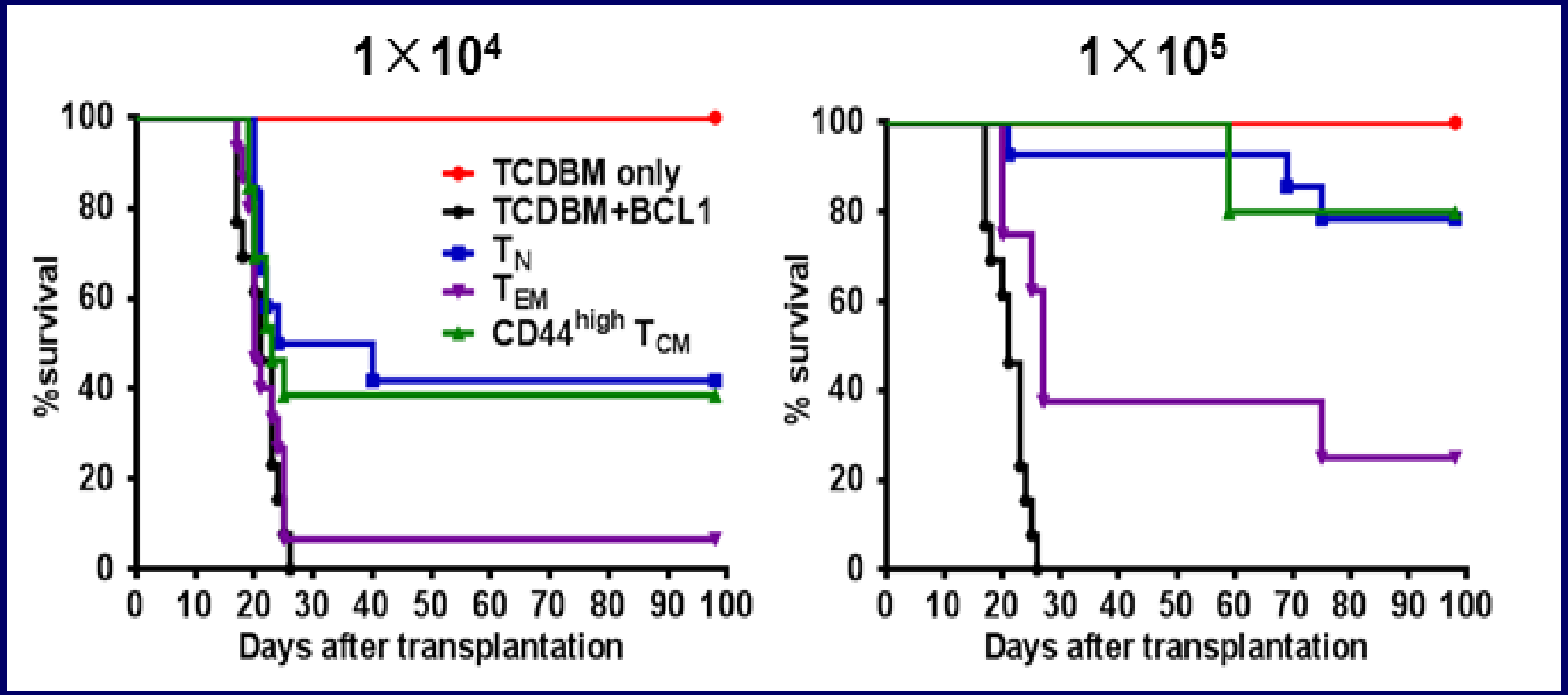
Recipient: **BALB/c 7 Gy**

*P<0.05, CD44^{high} T_{CM} vs.TCDBM only
&P<0.05, CD44^{high} T_{CM} vs.T_{EM}



CD44^{high} T_{CM} and T_N Mediate Comparable GVL Effect

Total



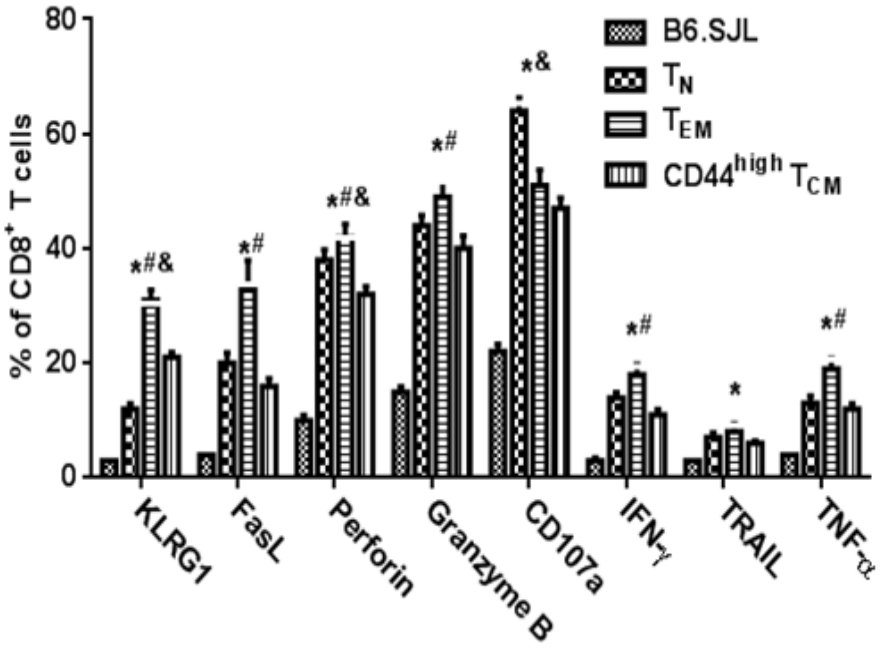
P= NS, CD44^{high} T_{CM} vs. T_N

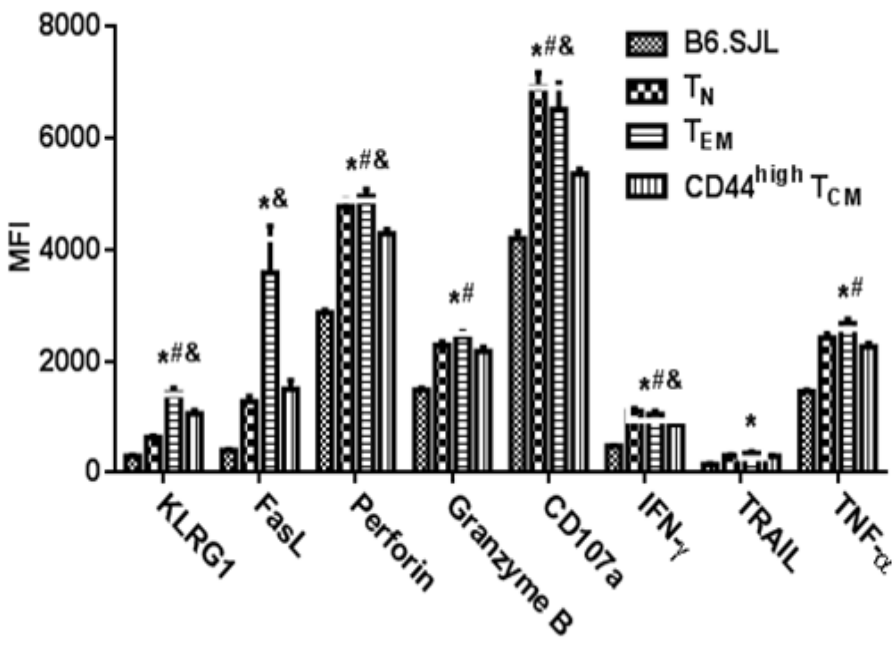
P<0.01, CD44^{high} T_{CM} or T_N vs. T_{EM} except at 1×10^4 (P=0.09)

P<0.05, CD44^{high} T_{CM} or T_N vs. TCDBM+BCL1 in all comparisons

$CD8^+$ $CD44^{high}$ T_{CM} Express Multiple Cytotoxic Molecules

Percentage **MFI**





*P<0.05, $CD44^{high}$ T_{CM} vs. B6.SJL
#P<0.05, $CD44^{high}$ T_{CM} vs. T_{EM}
& P<0.05, $CD44^{high}$ T_{CM} vs. T_N

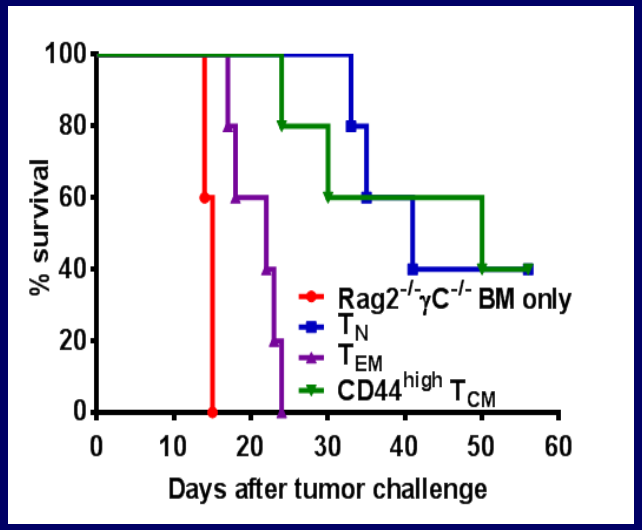
CD44^{high} T_{CM} Lose Alloreactivity Over Time upon Transfer into Allogeneic Recipients

Model

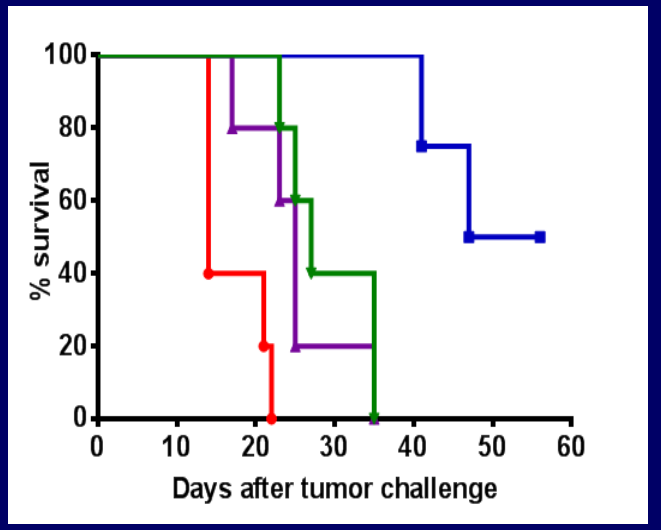
Graft:	**Rag2$^{-/-}$ γC$^{-/-}$ BMC**	**1x10^7**
	T cell subsets	**1x10^4**
Recipient:	**Nude BALB/c**	**8.5 Gy**

- **BCL1 cells: 5x10^5, i.p.**

Day



Day



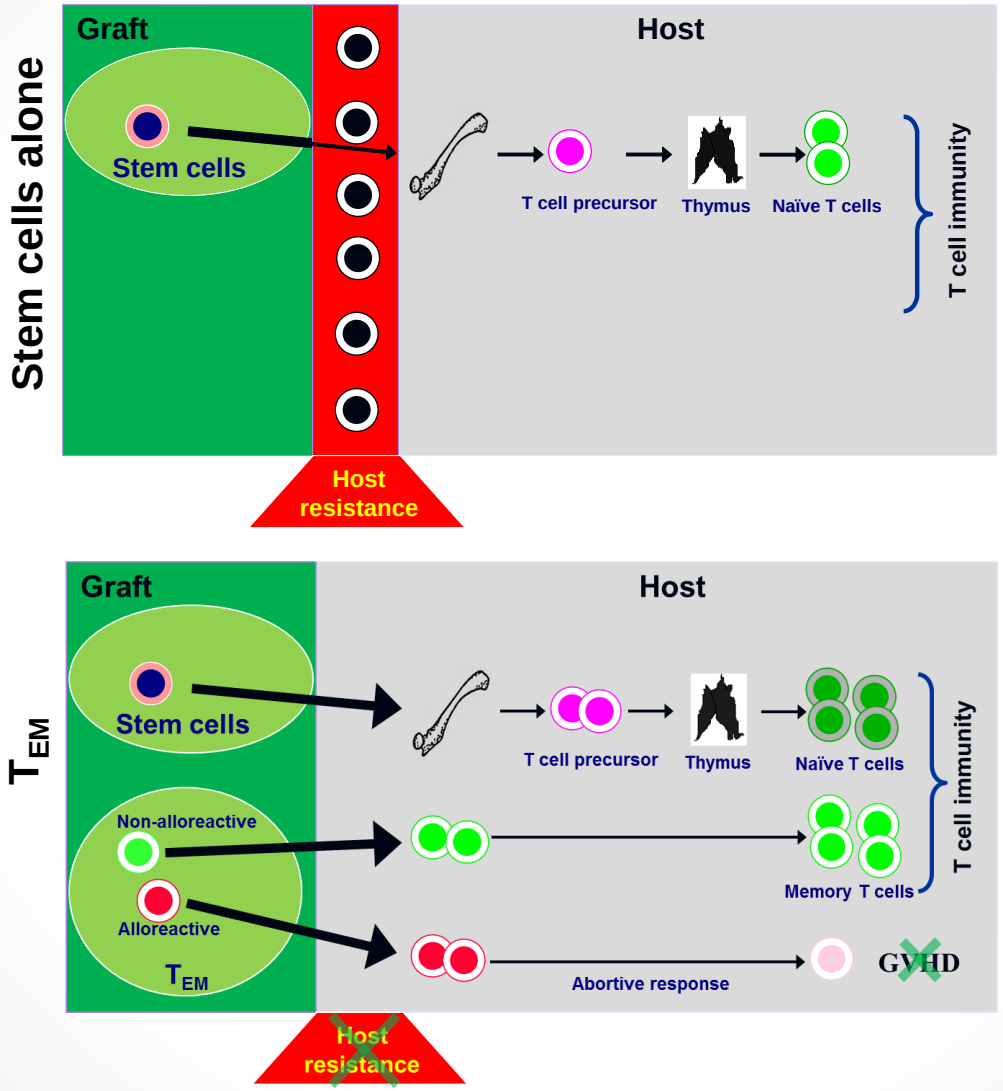

Stem cells alone
Graft
Host
Stem cells
T cell precursor
Thymus
Naïve T cells
T cell immunity
Host resistance
T EM
Graft
Host
Stem cells
Non-alloreactive
Alloreactive
T EM
T cell precursor
Thymus
Naïve T cells
Memory T cells
T cell immunity
Abortive response
GVHD
Host resistance

Figure 7

Conclusions II

- $CD44^{high}$ T_{CM} do not induce GVHD.
- $CD44^{high}$ T_{CM} = T_N in GVL and facilitation of engraftment.
- $CD44^{high}$ T_{CM} > T_{EM} in GVL and facilitation of engraftment.
- $CD44^{high}$ T_{CM} express multiple known cytotoxicity molecules.
- $CD44^{high}$ T_{CM} mediate an abortive alloresponse.

Clinical Trial

- One step selection using Miltanyi anti-CD45RA beads
- Dose escalation from 1 x 10e5/kg to 1 x 10e8/kg CD3+ cells in a 3 x 3 design
- Currently at dose level 5 x 10e7/kg
- No significant GVHD seen

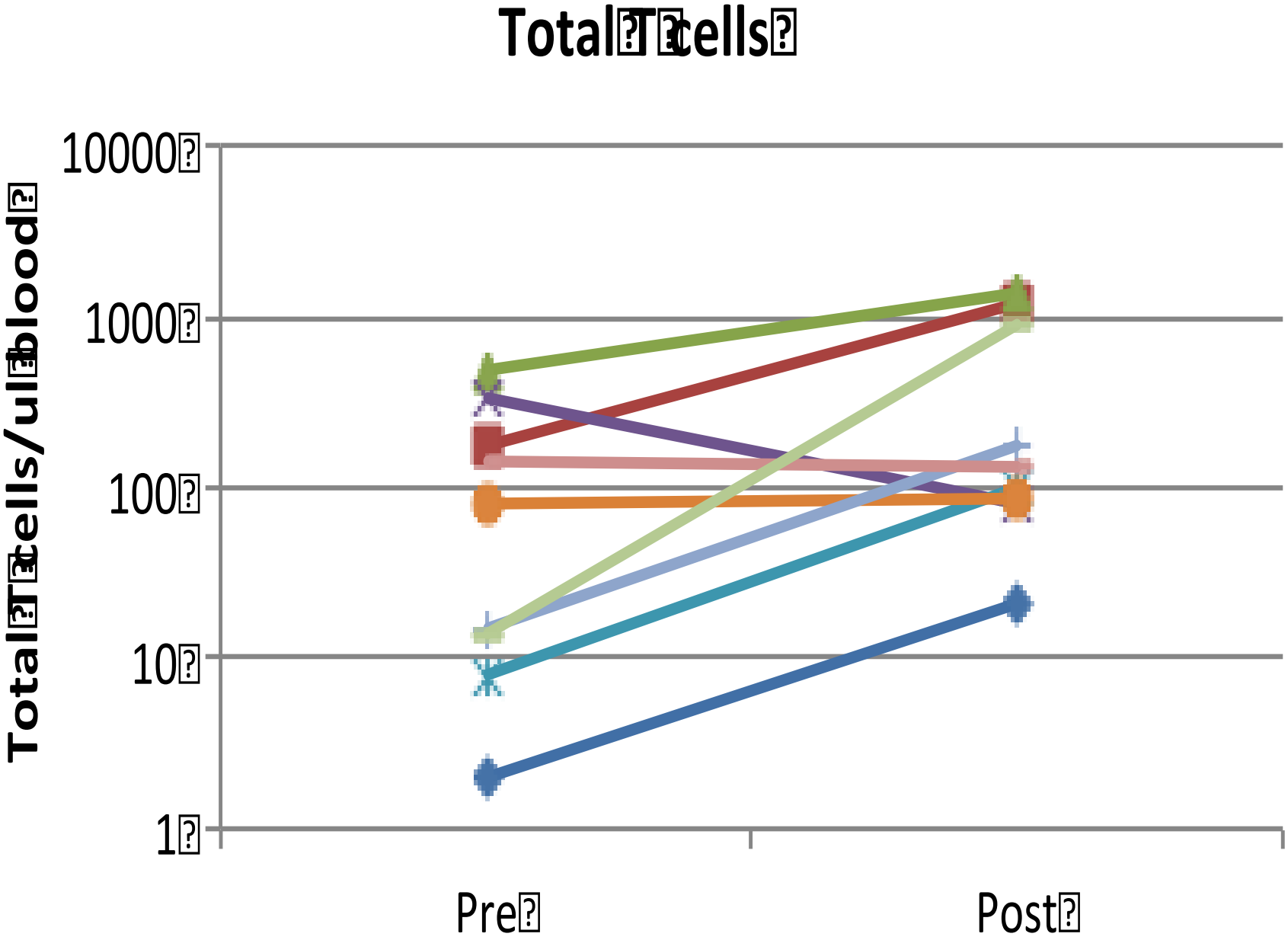
Clinical Trial

- **Inclusion Criteria**

- Patients who have undergone an alemtuzumab or thymoglobulin-containing allogeneic transplant procedure from an HLA-identical family donor, or an 8/8 HLA-matched unrelated donor. At least 60 days from day of transplantation
- Karnofsky performance status 50-100%.
- Donor myeloid engraftment (from peripheral blood or bone marrow) of at least 40% documented ≤ 60 days from protocol therapy
- No active acute GvHD ≥ grade II
- Prednisone (or equivalent corticosteroid) dose ≤ 20mg, daily mycophenolate mofetil dose ≤2000mg/d and cyclosporine/tacrolimus at ≤ therapeutic blood trough levels
- No change in dosing of immunosuppressive agents in the 2 weeks preceeding the naïve T-cell depleted donor lymphocyte infusion.
- No extensive chronic GvHD

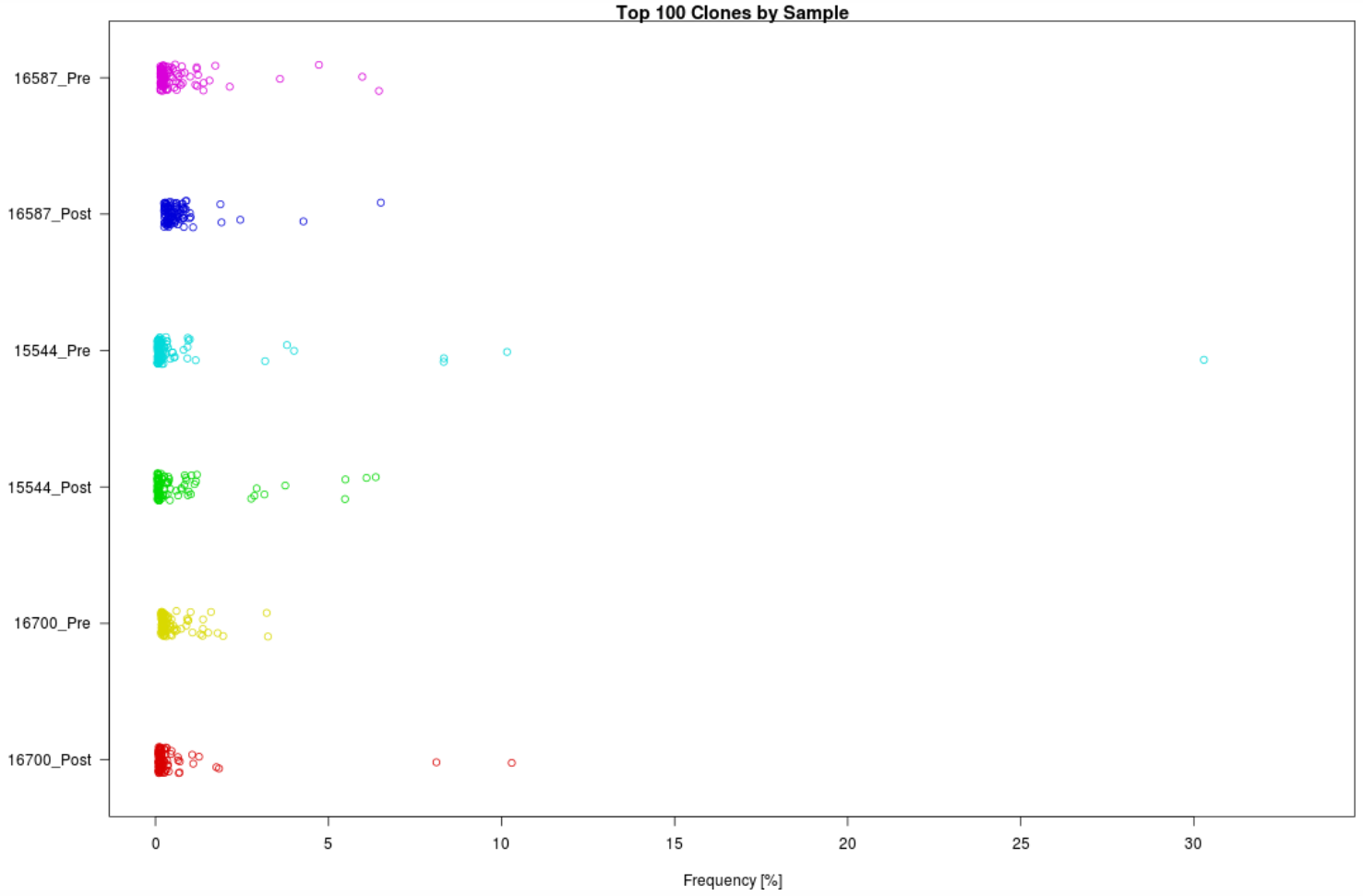
Patient No.	Age/sex	Diagnosis	Donor Source	CD45RA neg. DLI Dose	Graft vs. Host Disease		Clinical Outcome (months post DLI)
					Acute	Chronic*	
1	54/F	Multiple Myeloma	Matched Sibling	1 x 10^5/kg	--	--	Death/Relapse (3)
2	70/M	AML	Matched Unrelated	1 x 10^5/kg	--	--	Death/Relapse (3)
3	53/F	Multiple Myeloma	Matched Sibling	1 x 10^5/kg	--	--	Death/relapse (4)
4	30/M	Hodgkin Disease	Matched Sibling	1 x 10^6/kg	--	--	Death/relapse (6)
5	65/M	Non-Hodgkins Lymphoma	Matched Unrelated	1 x 10^5/kg	--	--	Death/Pancreatitis (5)
6	55/M	Non-Hodgkins Lymphoma	Matched Sibling	1 x 10^5/kg	Grade II (Skin)	--	Alive/CR (9)
7	65/M	CLL	Matched Unrelated	5 x 10^6/kg	Grade II (Stomach)	--	Alive/CR (7)
8	41/F	Non-Hodgkins Lymphoma	Matched Unrelated	5 x 10^6/kg	--	Moderate (lung)	Alive/CR (5)
9	65/M	Non-Hodgkins Lymphoma	Matched Sibling	5 x 10^6/kg	--	--	Alive/CR (4)
10	62/M	AML	Matched Sibling	1 x 10^7/kg			Alive/CR (2)
11	67/M	MDS	Matched Sibling	1 x 10^7/kg			Alive/CR (1)

*NIH consensus criteria

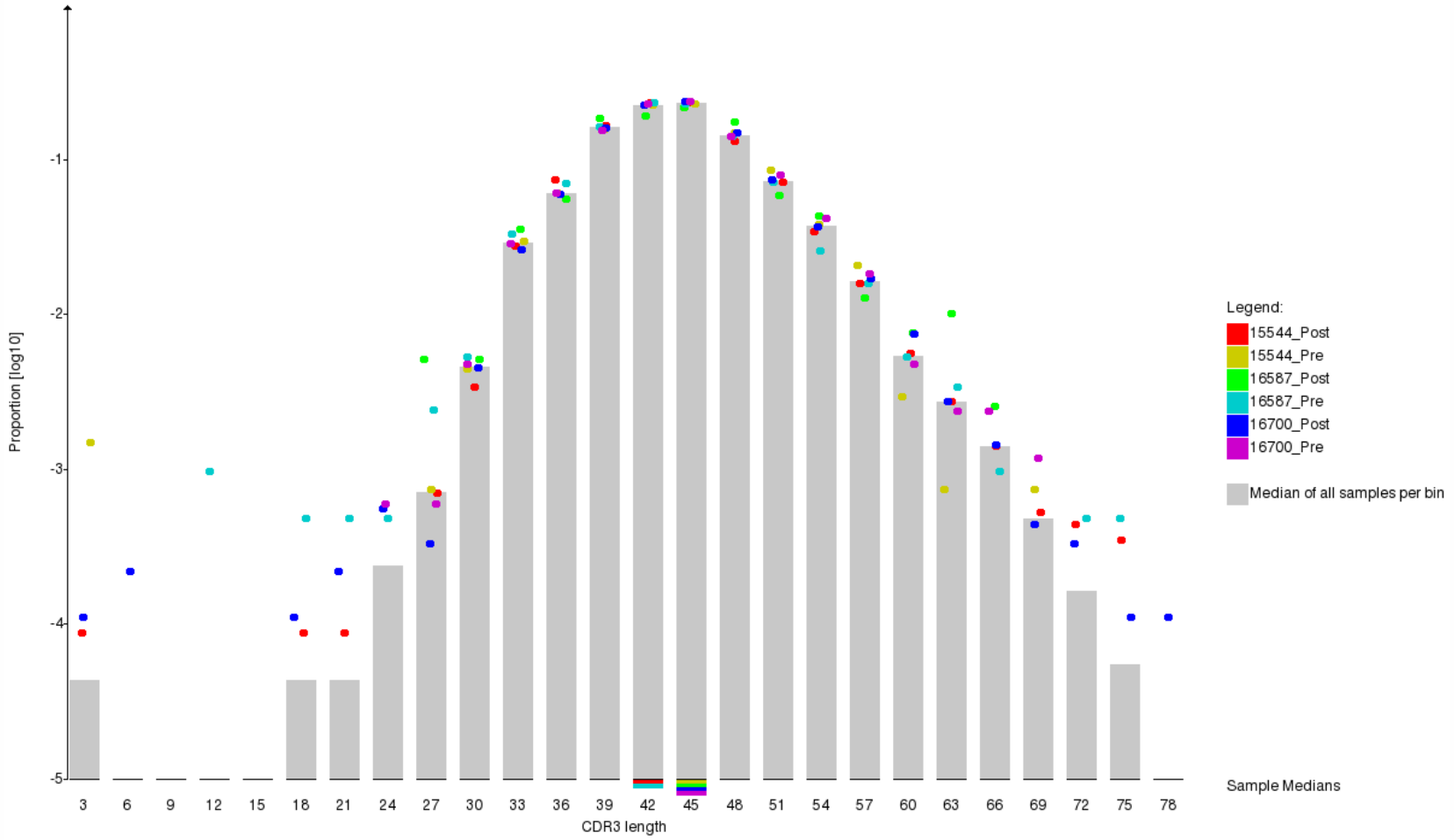

Total T cells
Total T cells/ul blood
10000
1000
100
10
1
Pre
Post

P = 0.04

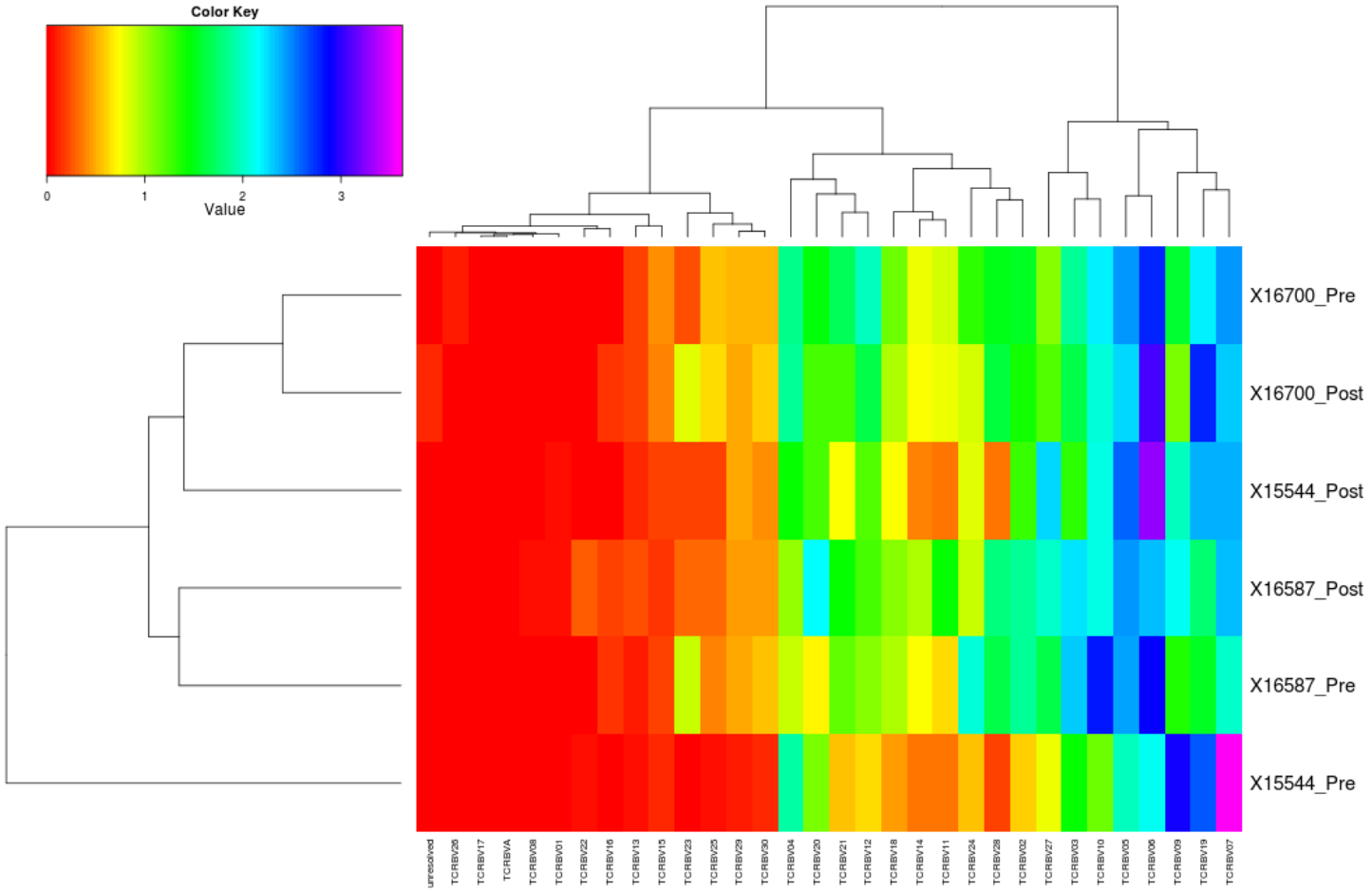
Frequencies of 100 most frequent clones per sample



CDR3 length distribution per sample



Heatmap of frequencies of v families per samples



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