SUPPLEMENTAL DATA for

Altered regulatory T-cell homeostasis in patients with CD4 lymphopenia following allogeneic hematopoietic stem cell transplantation

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Supplemental Figure 1,2.

Supplemental Table 1.

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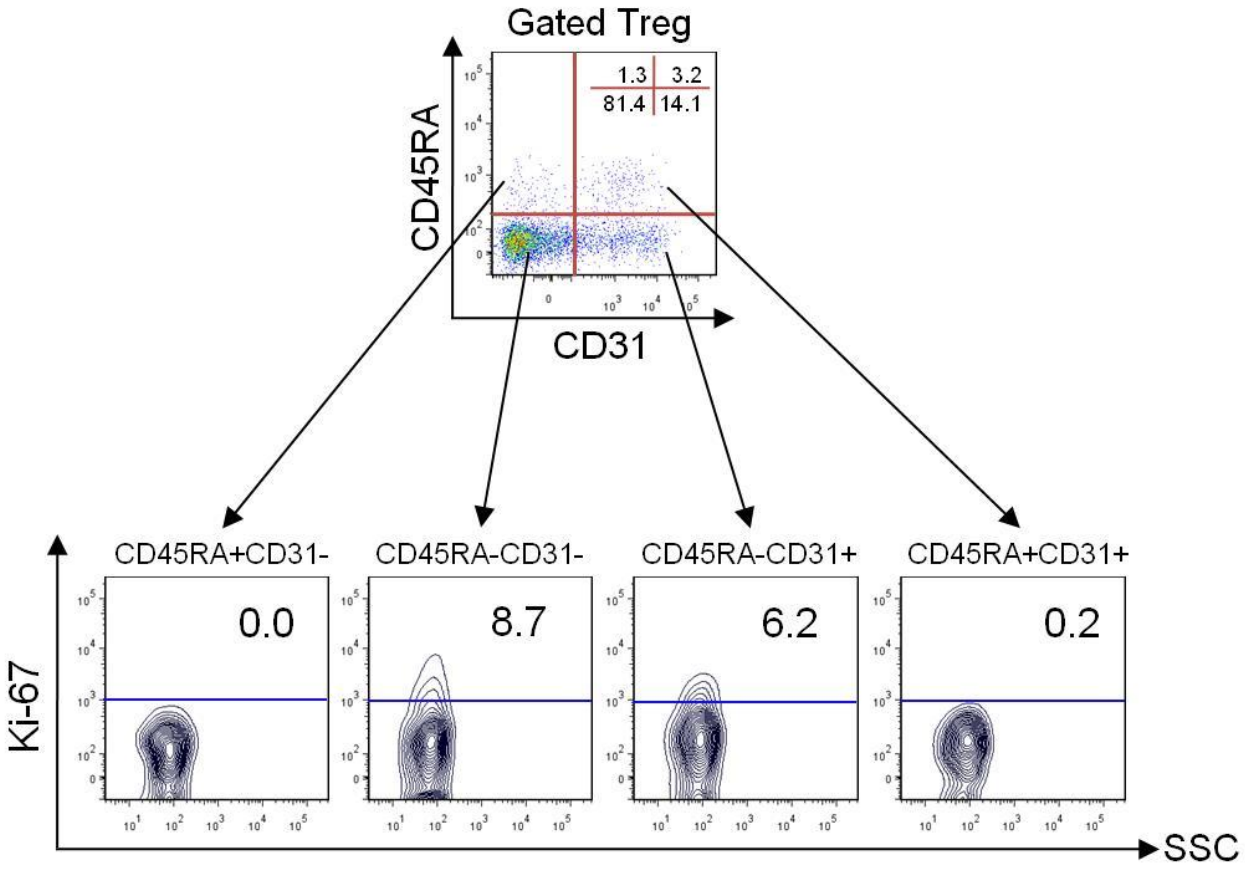
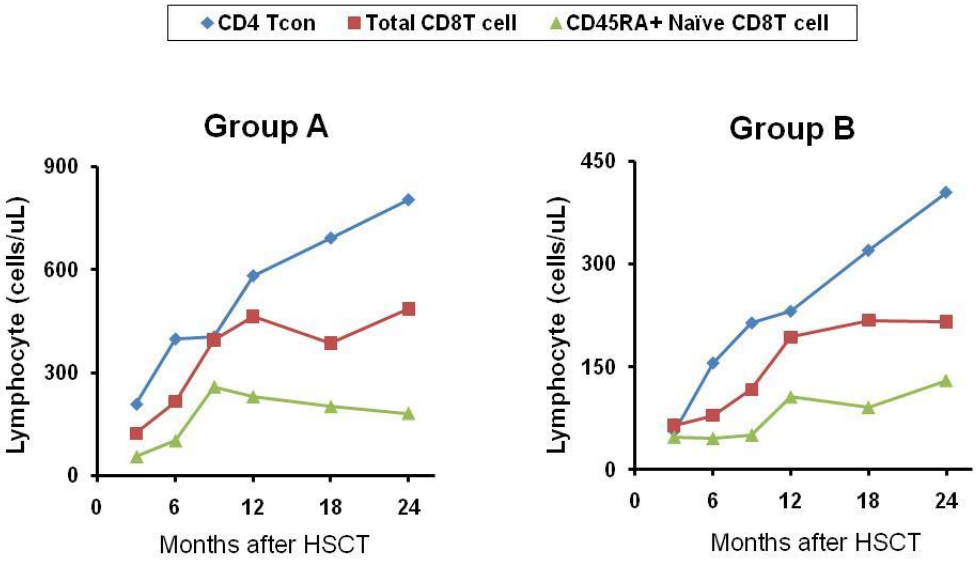
Supplemental Figure 1.



Supplemental Figure 1. CD45RA+CD31+ RTE Treg contain few Ki-67 expressing cells. PBMC were incubated with the following directly conjugated monoclonal antibodies for 20 minutes at 4°C: anti-CD4-Pacific Blue (clone RPA-T4; BD Biosciences), anti-CD25-PC7 (clone M-A251; BD Biosciences), anti-CD45RA-FITC (clone M-A251; Beckman Coulter), anti-CD127-APC-Alexa Fluor® 750 (clone eBioRDR5; eBioscience) and anti-CD31-APC (clone WM59; eBioscience). Surface-stained PBMC were processed using Fixation/Permeabilization buffer (BD Biosciences) and incubated with PE-conjugated anti-Ki-67 antibody (clone B56; BD Biosciences) for 30 minutes at room temperature. CD4 Treg defined as $CD4^{+}CD25^{med\text{-}high}CD127^{low}$ were further divided into 4 subpopulations by the expression of CD45RA and CD31. Expression of Ki-67 was determined on each Treg subset. Representative flow cytometry profiles from 11 post-HSCT patients are shown. *Top:* A representative panel for identification of 4 subpopulations based on the expression of CD45RA and CD31 is shown. *Bottom:* Ki-67 expression in 4 gated subpopulations of Treg are shown. RTE Treg are identified as $CD45RA^{+}CD31^{+}$. The blue line indicates the negative control for Ki-67 expression defined by isotype IgG control.

Supplemental Figure 2.



Supplemental Figure 2. Recovery of CD8 T cells and CD8 subsets in Cohort 1, Group A and B, during the 2 year observation period. The reconstitution of CD8 T cells was prospectively monitored and compared to CD4 Tcon. CD8 T cell subsets were defined using the following antibodies; anti-CD3-PC5 (clone UCHT1; Beckman Coulter), anti-CD8-PC7 (clone SFCI21Thy2D3; Beckman Coulter) and anti-CD45RA-FITC (clone M-A251; Beckman Coulter). Note the scale of Y-axis for Group B is half of the scale shown for Group A.

Supplemental Table 1

Characterization of hematopoietic stem cell products

	Cohort 1				Cohort 2				*P*-value
Surface Antigen	N	Median	Min	Max	N	Median	Min	Max	Cohort 1 vs 2
CD34	33	9.2×10^6	2.6×10^5	1.5×10^7	45	7.9×10^6	2.0×10^6	1.8×10^7	0.94
CD3	22	3.1×10^8	1.0×10^8	5.7×10^8	33	3.4×10^8	3.2×10^7	7.6×10^9	0.25
CD4	22	1.9×10^8	4.9×10^7	3.4×10^8	33	2.0×10^8	3.0×10^7	5.9×10^8	0.55
CD8	22	1.0×10^8	4.0×10^7	2.4×10^8	33	1.2×10^8	2.2×10^7	3.7×10^8	0.17
CD56	22	3.9×10^7	1.1×10^7	7.0×10^7	33	4.4×10^7	5.0×10^6	1.4×10^8	0.84
CD20	18	2.6×10^7	1.0×10^6	8.4×10^7	6	4.9×10^7	2.5×10^6	1.1×10^8	0.053

Values represent the median number of cells (/kg recipient weight) expressing corresponding surface antigens in the infused stem cell product for patients in Cohort 1 and 2.