

## CD56<sup>bright</sup> natural killer regulatory cells in filgrastim primed donor blood or marrow products regulate chronic graft-versus-host disease: the Canadian Blood and Marrow Transplant Group randomized 0601 study results

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<b>Supplemental Table 1. Logistic regression on all markers.</b>					
<b>Marker</b>	<b>Phenotyping</b>	<b>p-value</b>	<b>Median</b>	<b>Min</b>	<b>Max</b>
Total Lymphocyte count infused (x 10 <sup>8</sup> ) /Kg recipient		0.49	6.90	0.94	17.1
Total Lymphocytes infused (x 10 <sup>8</sup> )		0.25	1.70	0	6.4
% of Lymphocytes per CD45 <sup>+</sup>		0.35	25.1	8.9	55.0
Total # of CTL infused (x 10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup> CD8 <sup>+</sup>	0.35	0.27	0	1.6
% of CTL/total lymphocytes in donor product	CD3 <sup>+</sup> CD8 <sup>+</sup>	0.84	19.2	4.7	70.8
Total granzymeB <sup>+</sup> Perforin <sup>+</sup> CTL infused (x10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup> CD8 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.41	0.03	0	0.91
% of GranzymeB <sup>+</sup> Perforin <sup>+</sup> CTL/ total lymphocytes in donor product	CD3 <sup>+</sup> CD8 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.87	0.02	0.0003	0.7
% of GranzymeB <sup>+</sup> Perforin <sup>+</sup> CTL/total CTL	CD3 <sup>+</sup> CD8 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.79	10.40	0.20	56.1
Total # of Granzyme <sup>+</sup> Perforin <sup>+</sup> NK cells infused (x 10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup> CD56 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.61	0.06	0	0.74
% of Granzyme <sup>+</sup> Perforin <sup>+</sup> NK cells /total lymphocytes	CD3-CD56 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.63	0.04	0	0.32
% of Granzyme <sup>+</sup> Perforin <sup>+</sup> NK cells/total NK cells	CD3-CD56 <sup>+</sup> GranzymeB <sup>+</sup> Perforin <sup>+</sup>	0.16	55.00	3.50	96.0
Total number of CD56 <sup>bright</sup> cells infused /Kg recipient	CD3-CD56 <sup>bright</sup>	0.21	0.01	0	0.071
<b>%CD56<sup>bright</sup> NK cells (NK<sub>reg</sub>)/total lymphocytes</b>	CD3-CD56 <sup>bright</sup>	<b>0.003</b>	0.39	0.0199	2.83
Total number of NK <sub>reg</sub> cells infused (x 10 <sup>8</sup> )/Kg recipient	CD3-CD56 <sup>+</sup>	0.64	0.10	0	1.81
% of NK cells /total lymphocytes in donor product	CD3-CD56 <sup>+</sup>	0.94	7.20	2.20	78.0
Total B cells infused (x 10 <sup>8</sup> )/Kg recipient	CD19 <sup>+</sup>	0.21	0.22	0.018	1.40
% of B cells/Lymphocytes in donor product	CD19 <sup>+</sup>	0.36	13.35	2.25	38.1
<b>Total # of memory B-cells infused (x 10<sup>8</sup>)/Kg recipient</b>	CD19 <sup>+</sup> CD27 <sup>+</sup>	<b>0.03</b>	0.03	0	0.22
% of memory B cells /total lymphocytes in donor product	CD19 <sup>+</sup> CD27 <sup>+</sup>	0.14	2.00	0.37	10.1
Total # of Immature B cells infused (x 10 <sup>8</sup> )/Kg recipient	CD19 <sup>+</sup> CD27-IgD <sup>-</sup> CD10 <sup>+</sup>	0.84	0.02	0	0.088
% of immature B cells /total lymphocytes in donor product	CD19 <sup>+</sup> CD27-IgD <sup>-</sup> CD10 <sup>+</sup>	0.50	0.01	0.0019	0.073
Ratio of Immature to mature B cells	CD19 <sup>+</sup> CD27-IgD <sup>-</sup> CD10 <sup>+</sup> /CD19 <sup>+</sup>	0.10	0.004	0	0.06
Percentage of immature B cells/CD19 <sup>+</sup> B cells in donor product	CD27-IgD <sup>-</sup> CD10 <sup>+</sup> /CD19 <sup>+</sup>	0.23	7.85	1.70	45.70
Total # of T cells infused (x 10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup>	0.57	1.22	0.10	4.46
Total # of CD4 <sup>+</sup> T cells infused (x 10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup> CD4 <sup>+</sup>	0.79	1.32	0.11	3.98
% of CD4 <sup>+</sup> T cells /total lymphocytes in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup>	0.58	80.80	29.20	95.28
% of CD4 <sup>+</sup> T cells /CD3 <sup>+</sup> T cells in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup>	0.12	68.00	41.2	85.7
Total # of T <sub>reg</sub> cells infused (x 10 <sup>8</sup> )/Kg recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>hi</sup>	0.38	0.029	0.003	0.10
% of CD4 <sup>+</sup> CD25 <sup>hi</sup> T <sub>reg</sub> cells /total lymphocytes in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>hi</sup>	0.39	0.017	0.007	0.05

% of Treg cells /CD4 <sup>+</sup> CD25 <sup>hi</sup> T cells in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>hi</sup>	0.93	2.60	1.0	6.5
Total number of CD4 <sup>+</sup> FOXP3 <sup>+</sup> Treg cells infused/Kg recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> FOXP3 <sup>+</sup>	0.36	0.032	0.004	0.2
% of CD4 <sup>+</sup> FOXP3 <sup>+</sup> T <sub>reg</sub> cells /total lymphocytes in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup> FOXP3 <sup>+</sup>	0.10	0.022	0.007	0.05
% of CD4 <sup>+</sup> FOXP3 <sup>+</sup> / CD3 <sup>+</sup>	CD4 <sup>+</sup> FOXP3 <sup>+</sup>	0.20	3.00	1.00	7.00
Total CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>hi</sup> FOXP3 <sup>+</sup> T <sub>reg</sub> infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup>	0.36	0.038	0	0.15
% of CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>hi</sup> FOXP3 <sup>+</sup> per lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup>	0.14	0.005	0.0009	0.02
% of CD25 <sup>hi</sup> FOXP3 <sup>+</sup> per CD3 <sup>+</sup> CD4 <sup>+</sup>	CD25 <sup>hi</sup> FOXP3 <sup>+</sup> /CD3 <sup>+</sup> CD4 <sup>+</sup>	0.17	3.25	1.1	6.3
Total activated T <sub>reg</sub> infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup> /CD45RA <sup>-</sup>	0.38	0.05	0	0.2
% of memory T <sub>reg</sub> per lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup> /CD45RA <sup>-</sup>	0.14	0.007	0.001	0.02
% of memory T <sub>reg</sub> per CD3 <sup>+</sup> CD4 <sup>+</sup>	CD25 <sup>hi</sup> FOXP3 <sup>+</sup> CD45RA <sup>-</sup> /CD3 <sup>+</sup> CD4 <sup>+</sup>	0.23	4.15	1.0	7.7
Total naïve T <sub>reg</sub> infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup> /CD45RA <sup>+</sup>	0.65	0.006	0	0.03
% of naïve T <sub>reg</sub> per lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> /CD25 <sup>hi</sup> FOXP3 <sup>+</sup> /CD45RA <sup>+</sup>	0.51	0.004	0	0.03
% of naïve T <sub>reg</sub> per CD3 <sup>+</sup> CD4 <sup>+</sup>	CD25 <sup>hi</sup> FOXP3 <sup>+</sup> CD45RA <sup>+</sup> /CD3 <sup>+</sup> CD4 <sup>+</sup>	0.56	0.50	0.1	4.0
Total of memory T-cells infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD45RA <sup>-</sup>	0.40	0.65	0.06	3.0
% of memory T-cells per lymphocytes	CD3 <sup>+</sup> CD45RA <sup>-</sup>	0.65	34.50	14.2	73.4
Total of memory T-cells infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD45RA <sup>-</sup>	0.39	0.50	0.04	2.49
% of memory CD4 <sup>+</sup> per lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RA <sup>-</sup>	0.91	39.90	17.7	62.0
Total of Th1 cells infused (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> IFNγ <sup>+</sup>	0.61	0.014	0	0.3
% of Th1 cells /total lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> IFNγ <sup>+</sup>	0.11	0.013	0.0000	0.08
<b>% of CD4<sup>+</sup>IFNγ<sup>+</sup> cells / total CD3<sup>+</sup></b>	<b>CD4<sup>+</sup>IFNγ<sup>+</sup>/CD3<sup>+</sup></b>	<b>0.002</b>	8.34	0.01	30.0
Total of Th2 (x10 <sup>8</sup> ) /Kg of recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-4 <sup>+</sup>	0.94	0.009	0.0003	0.08
% of Th2 per lymphocytes	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-4 <sup>+</sup>	0.56	0.006	0.0005	0.04
% of CD4 <sup>+</sup> IL-4 <sup>+</sup> per CD3 <sup>+</sup>	CD4 <sup>+</sup> IL-4 <sup>+</sup> /CD3 <sup>+</sup>	0.42	0.96	0.08	6.0
% of Th17 T cells infused (x 10 <sup>8</sup> ) /Kg recipient	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17 <sup>+</sup>	0.86	0.004	0.0006	0.09
% of Th17 T cells infused /total lymphocytes in donor product	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17 <sup>+</sup>	0.23	0.003	0.0003	0.01
% of CD4 <sup>+</sup> IL-17 <sup>+</sup> per CD3 <sup>+</sup>	CD4 <sup>+</sup> IL-17 <sup>+</sup> /CD3 <sup>+</sup>	0.12	0.42	0.05	2.00
Total number of activated B cells infused (x10 <sup>8</sup> ) /Kg recipient	CD19 <sup>+</sup> CD21 <sup>lo</sup>	0.35	0.01	0	0.11
% of activated B cells /total lymphocytes in donor product	CD19 <sup>+</sup> CD21 <sup>lo</sup>	0.23	0.007	0.0009	0.04
Activated B cells /total CD19 <sup>+</sup>	CD19 <sup>+</sup> CD21 <sup>lo</sup> /CD19 <sup>+</sup>	0.37	2.80	0.30	13.5
% of TLR9 <sup>hi</sup> B cells infused (x 10 <sup>8</sup> ) /Kg recipient	CD19 <sup>+</sup> TLR9 <sup>hi</sup>	0.23	0.007	0.0000	0.07
% of TLR9 <sup>hi</sup> B cells /total lymphocytes in donor product	CD19 <sup>+</sup> TLR-9 <sup>hi</sup>	0.14	0.003	0.0003	0.02
TLR9 <sup>hi</sup> B cells in total CD19 <sup>+</sup>	CD19 <sup>+</sup> TLR-9 <sup>hi</sup> /CD19 <sup>+</sup>	0.08	1.20	0.23	5.40
% of iNKT out of lymphocytes		0.32	3.3	0.3	30.1
Total # of iNKT infused /Kg		0.92	0.045	0.001	0.29

<b>Supplemental Table 2. Association between Donor Cell Population Numbers with Development of Chronic GvHD (Includes all patients that has a death or relapse before the onset of cGvHD or 2 years of follow up)</b>		
<b>Percentage cells per total lymphocytes – Independent variable</b>		
<b>Variable</b>	<b>P-value (Wald test)</b>	<b>Hazard Ratio (95% CI)</b>
CD56 <sup>bright</sup> NK cells (N = 107)		
Overall cGvHD	0.02	0.54 (0.32-0.90)
Extensive cGvHD	0.02	0.54 (0.33-0.90)
CD4 <sup>+</sup> T cells IFN $\gamma$ (N = 100)		
Overall cGvHD	0.001	0.93 (0.89-0.97)
Extensive cGvHD	0.007	0.95 (0.91-0.99)

<b>Supplemental Table 3. Impact of Clinical Variables on interpretation of Donor Cell Population Populations and Development of Chronic GvHD – All evaluable patients</b>		
<b>CD56<sup>bright</sup> NK cell marker</b>		
<b>Variable</b>	<b>P-value (Wald test)</b>	<b>Odd Ratio (95% CI)</b>
Female donor>male recipient vs. others	0.52	0.70 (0.23-2.08)
TBI versus no TBI	0.43	1.72 (0.44-6.61)
Recipient age > median vs. ≤ median	0.71	0.85 (0.37-1.97)
Donor age > median vs. ≤ median	0.61	0.80 (0.35-1.86)
AML vs. all other diagnoses	0.87	0.93 (0.41-2.14)
Transplant related mortality	0.80	0.89 (0.35-2.2)
Relapse	0.97	1.02 (0.40-2.6)
<b>CD4<sup>+</sup> T cells IFN<math>\gamma</math></b>		
<b>Variable</b>	<b>P-value (Wald test)</b>	<b>Odd Ratio (95% CI)</b>
Female donor>male recipient vs. others	0.98	1.00 (0.92-1.08)
TBI versus no TBI	0.66	1.03 (0.90-1.17)
Recipient age > median vs. ≤ median	0.90	1.00 (0.94-1.07)
Donor age > median vs. ≤ median	0.74	1.01 (0.94-1.08)
AML vs. all other diagnoses	0.52	0.98 (0.92-1.05)
<b>Transplant related mortality</b>	<b>0.01</b>	<b>0.89 (0.81-0.98)</b>
Relapse	0.75	0.99 (0.91-1.07)

TBI = total body irradiation, AML = Acute Myeloid Leukemia