Supplemental Appendix to:

Reduced intensity conditioning and single unit unrelated cord blood transplant with optional immune boost for non-malignant disorders

Mark T. Vander Lugt, MD[1], Xiaohua Chen, MD[1], Maria.L. Escolar, MD[2], Beth A. Carella, DO[1], Jessie L. Barnum, MD[1], Randy M. Windreich, MD[1], Memphis J. Hill, BS, [1], Rebecca A. Marsh, MD[3], Heather Stanczak, MT(ASCP)[1], Elizabeth O. Stenger, MD[1], and Paul Szabolcs, MD[1]

[1]Division of Blood and Marrow Transplantation and Cellular Therapies, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA 15116; [2]Neurodevelopment in Rare Diseases Program, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA 15116; [3]Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

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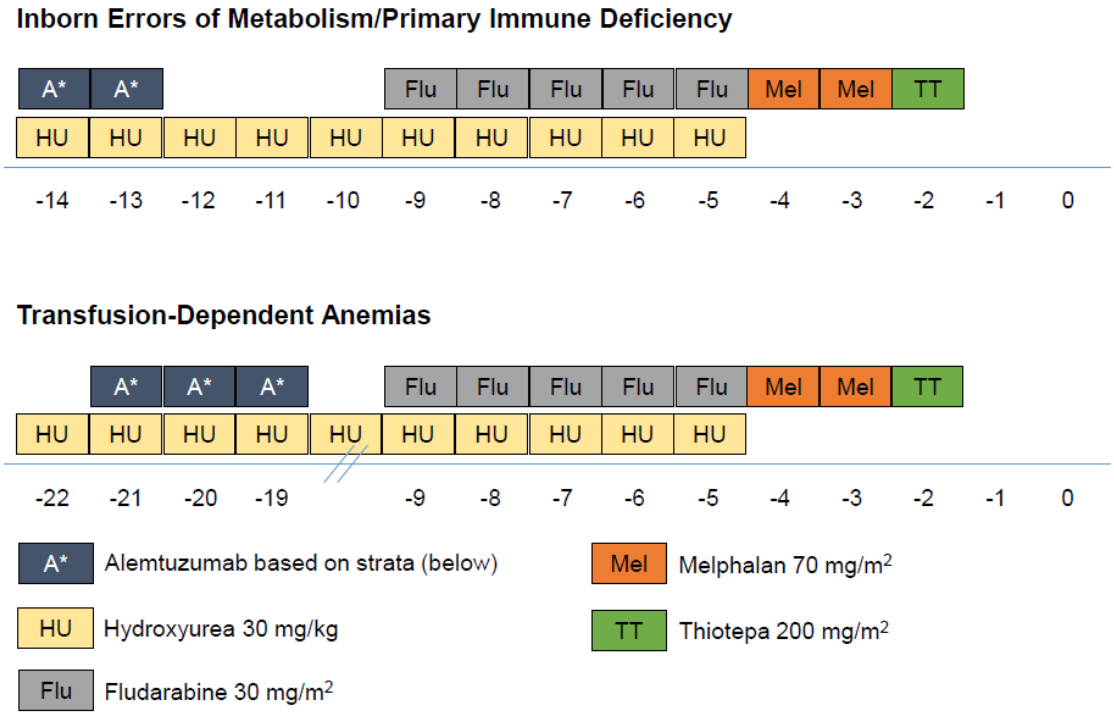
Figure S1. Conditioning regimen and alemtuzumab strata.



Stratum 1: 1 mg/kg (max dose 30 mg) alemtuzumab per dose		
Age	**ALC**	
< 2 years	> 2000	Absence of significant infection
≥ 2 years	> 1000	Absence of significant infection
Stratum 2: 0.5 mg/kg (maximum dose 15 mg) alemtuzumab per dose		
Age	**ALC**	
< 2 years	> 2000	Presence of significant infection
	1000-2000	Absence of significant infection
≥ 2 years	> 1000	Presence of significant infection
	500-1000	Absence of significant infection
Stratum 3: No treatment dose of alemtuzumab (test dose only)		
Age	**ALC**	
< 2 years	1000-2000	Presence of significant infection
	< 1000	Absence of significant infection
≥ 2 years	500-1000	Presence of significant infection
	< 500	Absence of significant infection

Figure S2. Neurocognitive outcomes for symptomatic infantile-onset Krabbe disease. Cognitive outcomes are shown by plotting age-equivalent score (developmental age) against actual age (calendar age) for symptomatic infantile Krabbe patients. Red and blue lines indicate individual values of children who received RIC (red) and historical MAC (blue). Gray lines represent the mean and approximate variability (95% CI) observed in the normal population.

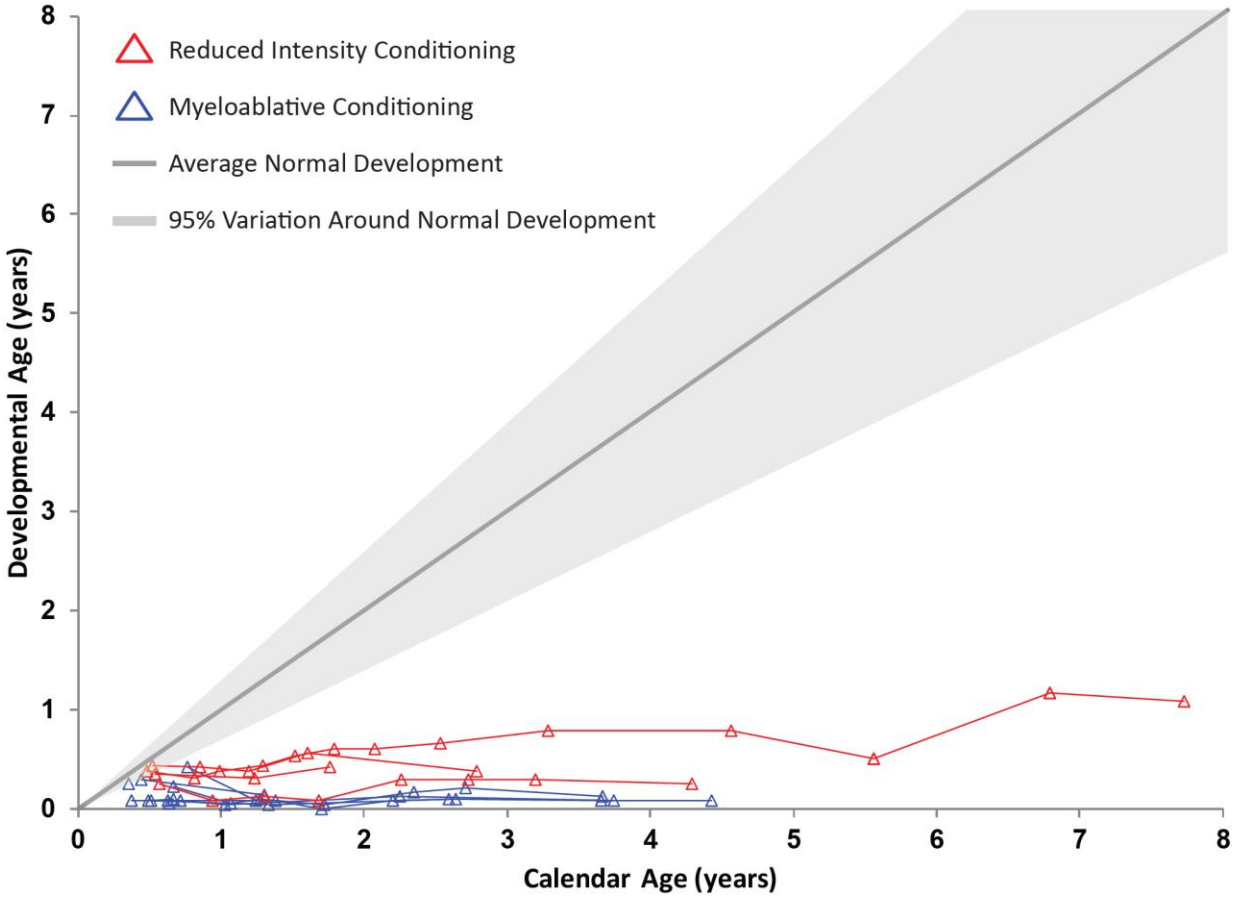


Figure S3. **Immune Reconstitution post-transplant.** Immune reconstitution measured as the percent of age-appropriate normal values of each lymphocyte subset achieved at each time point. Normal values of lymphocyte subsets were defined as an absolute cell count of ≥10th percentile of those seen in healthy children of the same age at the time of measurement as defined in Shearer et al.[1] At 3 months post-transplant, CD3, CD4, CD8, CD4/CD45RA/CD62L+ (surrogate recent thymic emigrants (RTE)) and NK cell numbers were found in 0%, 0%, 0%, 0% and 81.3% of 43 patients, respectively. At 6 months, normal, age-adjusted CD3, CD4, CD8, RTE and NK cell numbers were found in 10.3%, 15.4%, 15.3%, 19.4% and 82.1% of 39 patients, respectively. At 1 year, normal, age-adjusted CD3, CD4, CD8, RTE and NK cell numbers were found in 68.6%, 71.4%, 60.0%, 71.0% and 85.7% of 35 patients, respectively.

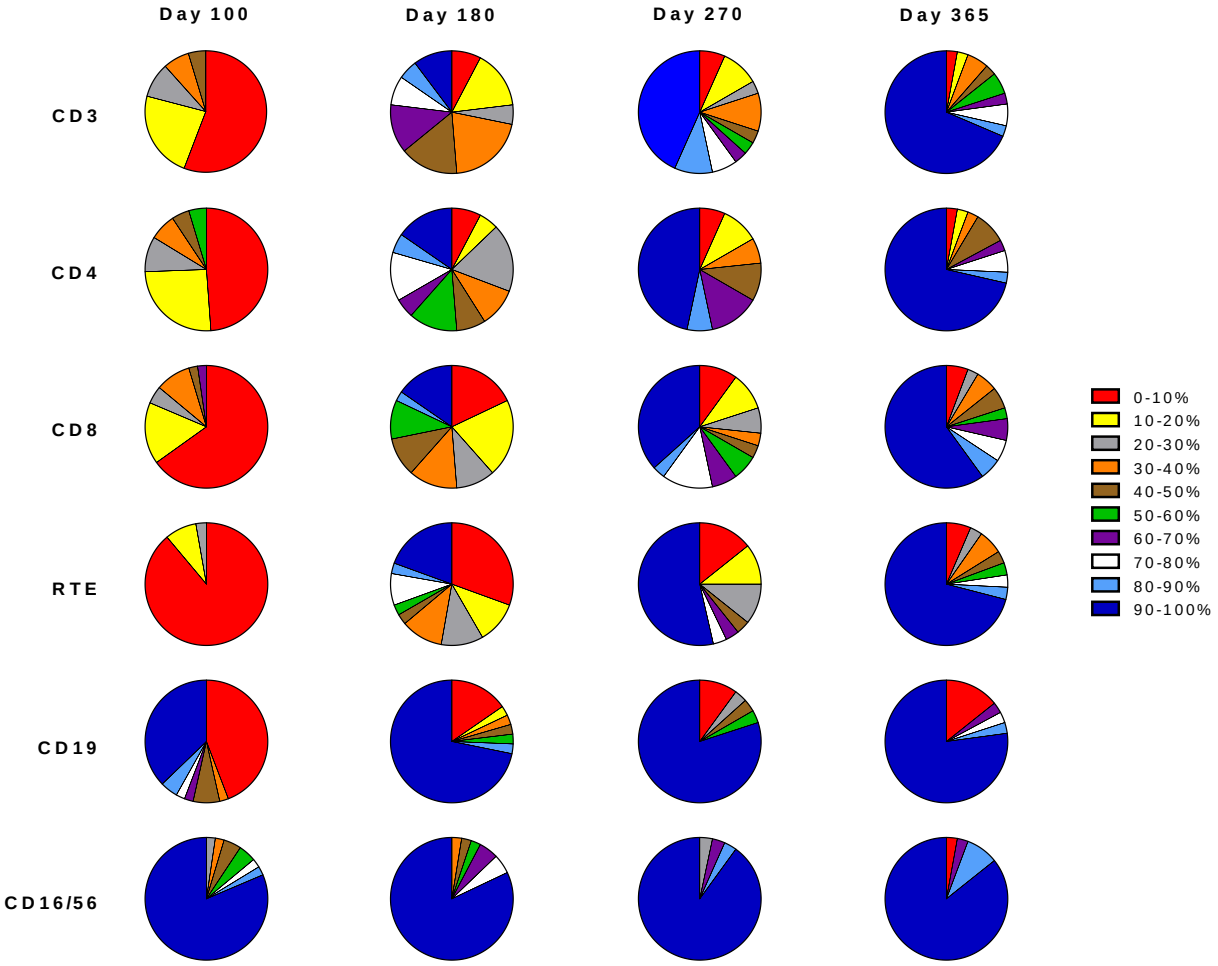


Figure S4. Infection post-transplant. The cumulative incidence of first infection after initiation of conditioning was 61% (95% CI: 49-72%) and the first infection was detected at a median of 18 days post-transplant (range: -27-224) post-transplant. Adenovirus was the most commonly seen infections, occurring in 11 subjects, followed by EBV, HHV-6, and CMV in 10, 9, and 6 subjects, respectively. 2 subjects develop non-viral opportunistic infections with *Mycobacteria kansasii* or *Candida parapsillosis.* 8 subjects had infection with more than one organism.

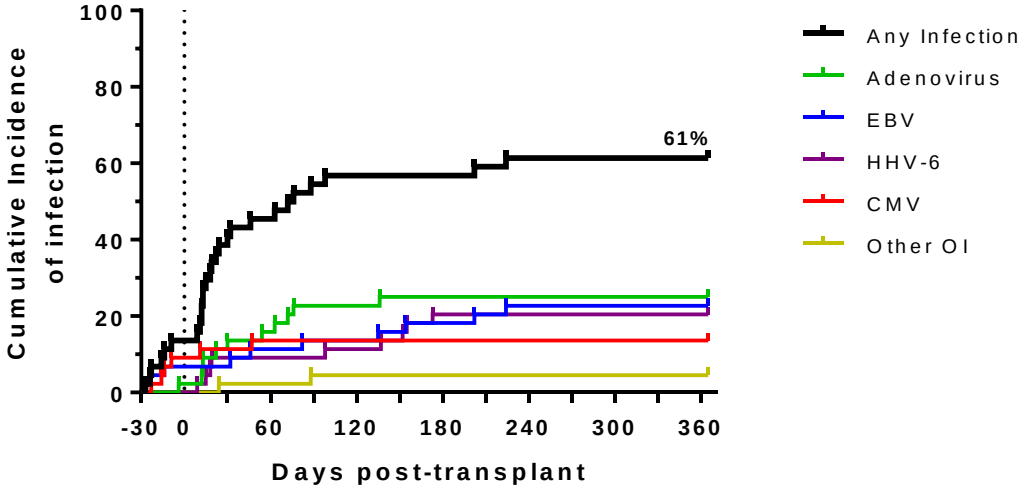


Figure S5. Correlation of alemtuzumab level with GVHD, infection and chimerism. (A) The cumulative incidence of grade II-IV GVHD in patients with low day 0 alemtuzumab was 23% compared to 15% in those with high day 0 alemtuzumab levels (p=0.5). (B) The cumulative incidence of first viral infection in patients with both low and high day 0 alemtuzumab levels was 54% (p-0.9). (C) The median donor percentage in patients with low and high day 0 alemtuzumab levels was 100% donor in both whole blood and T cell fractions (p=0.35 and p=0.94, respectively).

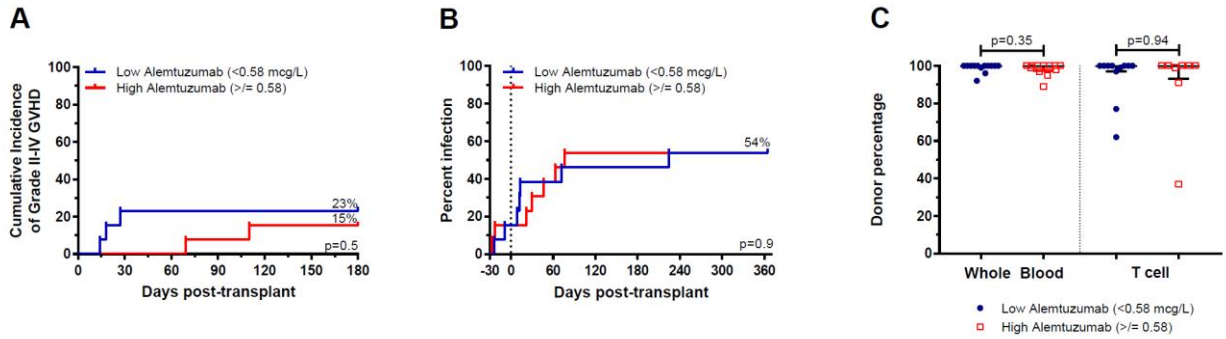


Figure S6: Day of cDLI post-transplant and Kinetics of Immune reconstitution. (A) The distribution of the day of cDLI infusion post-transplant is shown here. The median day of infusion was day 66 post-transplant. Error bars indicate the interquartile range. (B-D) The kinetics of T cell reconstitution for subjects who received cDLI between day 38 and 75 post-transplant (N=14) are shown in red, while those who never received cDLI (N=24) are shown in blue. The inset shows the absolute T cell numbers at day 60 and 100 post-transplant. All points represent median absolute values with error bars indicating the interquartile range. The lines between points represent the best-fit line using simple linear regression. No significant difference was found between rate of increase in absolute T cell numbers between those who received cDLI and those who did not receive cDLI.

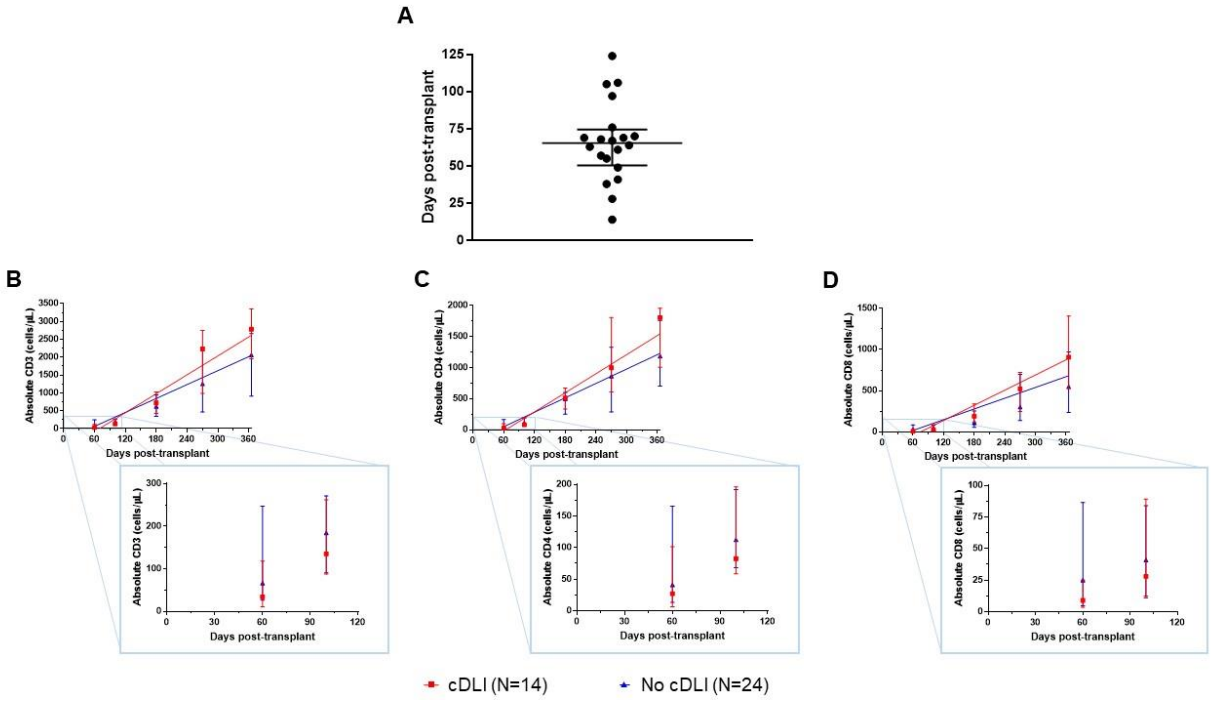


Table S1. Comparison between Retrospective and Prospective Patients.

Patient Characteristics		**Retrospective N=15**	**Prospective N=29**	**p-value**
Age (yrs). – median (range)		1 (0.5-14)	3 (0.4-16.6)	0.19
Weight (kg) – median (range)		10 (7-52)	15 (6-74)	0.33
Gender – no (%)	Male	8 (53%)	19 (66%)	0.52
Diagnosis – no (%)	IMD	8 (53%)	22 (76%)	0.07
	PID	6 (40%)	3 (10%)	
	Other	1 (7%)	4 (14%)	
Graft Characteristics				
HLA Match (of 6)	6	7 (47%)	9 (31%)	0.28
	5	4 (27%)	15 (52%)	
	4	4 (27%)	5 (17%)	
HLA Match (of 8)	8	4 (27%)	4 (14%)	0.52
	7	2 (13%)	8 (28%)	
	6	4 (27%)	10 (34%)	
	≤5	5 (33%)	7 (24%)	
TNC/kg (x10^7) – median (range)		9.6 (3.8-20.8)	8.4 (2.3-24.8)	0.92
CD34/kg (x10^5) – median (range)		2.59 (1.55-6.17)	3.59 (0.92-9.24)	0.29
Gender mismatch – no (%)		7 (47%)	15 (52%)	>0.99
ABO mismatch – no (%)		11 (73%)	18 (62%)	0.52
Transplant Outcomes				
Day Neutrophil engraftment – median (range)		15 (12-33)	15 (10-21)	0.80
5-year overall survival – % (95% CI)		87% (56-97%)	68% (46-90%)	0.75
5-year event-free survival – % (95% CI)		87% (56-97%)	66% (25-88%)	0.56
Transplant complications				
Acute GVHD (Grade I-IV) – cumulative incidence (95% CI)		40% (13-67%)	52% (35-71%)	0.49
GVHD (Grade I-IV) onset day – median (range)		40 (13-145)	69 (13-139)	0.89
Acute GVHD (Grade II-IV) – cumulative incidence (95% CI)		33% (7-64%)	24% (12-44%)	0.58
GVHD (Grade II-IV) onset day – median (range)		47 (21-145)	28 (14-110)	0.43
Opportunistic Infections at 1 year – cumulative incidence (95% CI)		50% (22-72%)	60% (42-79%)	0.67

REFERENCES:

1. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. The Journal of allergy and clinical immunology 2003;112:973-80.