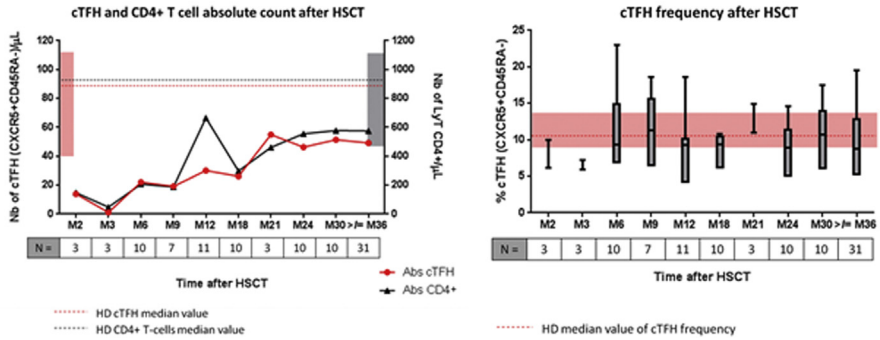

Figure 1. (Left) Recovery of tTFH and CD4+ T-cells/µL) after HSCT. (Right) Frequencies of cTFH in the CD4+ T-cell gate beginning 2 months after HSCT. Number of patients analyzed for each time point is indicated below the graph. Dashed lines represent the median values from 11 Healthy Donors (90% CI plotted in light red and light grey for the respective population).

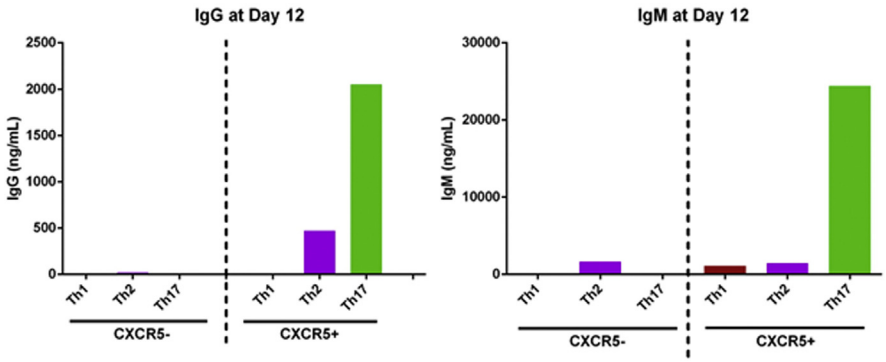

Figure 2. Assessment of B-tell helper function in cTFH subsets. cTFH (CD4+CD45RA-CXCR5+) and non-cTFH (CD4+CD45RA-CXCR5-) T cell subsets were purified by flow cytometric cell sorting. IgG (left) and IgM (Right) production was measured in supernatants? after 12-day co-culture of purified T-cell subsets with naive B-cells stimulated with Staphylococcal Enterotoxin B.

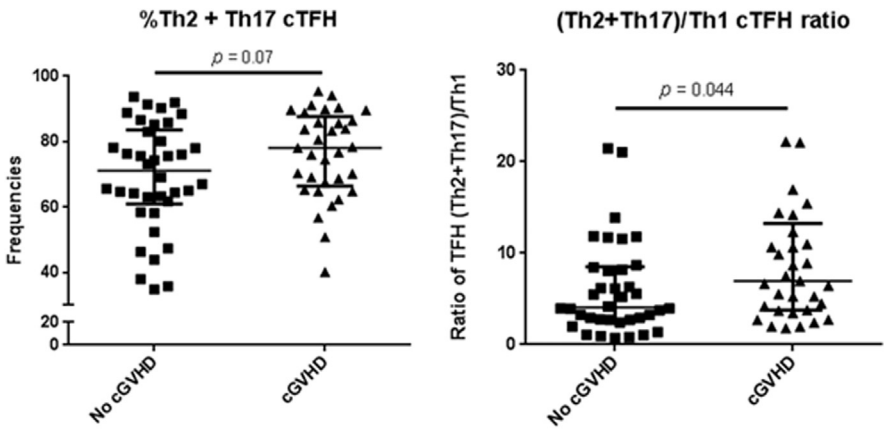

Figure 3: Comparison of Th2+Th17 cTFH (*Left*) and (Th2+Th17)/Th1 cTFH ratio (*Right*) in patients without cGVHD (N=38) and with cGVHD (N=33).

cGVHD patients, we observed a selective decrease of the Th1 compartment in the cTFH subset (16.2% and 10.9% for No *vs.* cGVHD, *p=0.034*), and relative increase of both Th2 and Th17 cTFH (Th2+Th17= 71.15 and 78.1% respectively, *p=0.07*) leading to a greater (Th2+Th17)/Th1 ratio (*p=0.044*) (Figure 3). We did not find any differences in cTFH subsets when comparing Mild *vs.* Moderate and Severe cGVHD. To further characterize cTFH, we analyzed BCL-2 and CD95 apoptosis pathways. Th2 and Th17 cTFH subsets appeared to express lower levels of CD95 and higher levels of BCL-2 compared to Th1 cTFH.

Increased representation of Th2 and Th17 cTFH subsets that are relatively resistant to apoptosis may be a mechanism that promotes B-cell deregulation and pathologic antibody production in patients with cGVHD. Targeting Th2/Th17 cTFH or TFH-B cell interactions may provide novel therapies in patients with cGVHD.

204

CD3/8 T-Cell Responses to CMV Reactivation and Association with Overall Survival in T-Cell Replete Haploidentical Transplants: A Retrospective Analysis

Mahasweta Gooptu [1], Benjamin Leiby [2], Onder Alpdogan [3], Matthew Carabasi [3], Joanne Filicko [4], Margaret Kasner [3], Thomas Klumpp [1], Ubaldo Martinez [5], Barbara Pro [3],

Manish Sharma [6], John L. Wagner [5], Mark Weiss [3], Neal Flomenberg [3], Dolores Grosso [3]. *[1] Medical Oncology, Thomas Jefferson University Hospital, Philadelphia, PA; [2] Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA; [3] Medical Oncology, Thomas Jefferson University, Philadelphia, PA; [4] Thomas Jefferson University Hospital, Philadelphia, PA; [5] Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; [6] Medical oncology, Thomas Jefferson University, Philadelphia, PA*

CMV reactivation has been associated with increased non-relapse mortality (NRM) and improved early relapse incidence (RI) post HLA matched allogeneic hematopoietic stem cell transplant (HSCT), however, it's effect has been less extensively studied in T cell replete haploidentical (HI) HSCT. In the 2 step approach to HI HSCT developed at our institution, a large fixed dose of T cells (2×10^8) is administered after conditioning (Step 1), followed 2 days later by cyclophosphamide (CY) for T cell tolerization. In Step 2, a CD34-selected stem cell product is infused 1 day after completing CY. A significant increase in T cell numbers, especially in CD3/8 counts, was associated with CMV reactivation in many patients treated with this approach. We hypothesized that a CMV-associated increase in CD3/8 counts would impact HSCT outcomes.

A retrospective outcomes analysis (OS, NRM and RI) using multivariable proportional hazards regression was performed on all patients enrolled on a 2 step clinical trial since 2006, who were alive and disease free at D90 (n=106). High v low CD3/8 count at D90, a history of GVHD treated with steroids and CMV reactivation (defined by > 100 copies/ml by PCR) both by D90, were the factors of interest. Known predictors of outcomes including disease at HSCT and hematopoietic comorbidity index (HCT CI) were included in the analysis. The median CD3/8 count for the group, 125 cells/ul, was used to differentiate CD8H v CD8L levels.

43% patients reactivated CMV prior to D90. The median CD3/8 count for CMV-R (reactivators) v CMV-NR (non-reactivators) was 308.4 v 53.7 cells/ul ($p<0.0001$). For the whole group, CD8H had a significant protective effect for OS and NRM. CMV reactivation, disease at HSCT and higher HCT CI score had a significant negative impact. Table 1. No variables were significantly associated with RI. In a subset analysis, patients with acute GVHD/CD8H had superior OS in both CMV-R and CMV-NR groups. CMV-NR patients with CD8L/no acute GVHD had the poorest OS. Fig 1. CMV-R patients with CD8L had equally poor OS with or without acute GVHD. Fig 2.

Higher CD3/8 counts were significantly associated with improved OS and lower NRM in all patients irrespective of CMV reactivation. Higher CD3/8 counts in CMV-R patients may mitigate the effects of CMV reactivation while preserving the beneficial effects of GVHD on OS, a finding that requires further investigation. Prospective analyses of CD3/8 and CD3/4 numbers and strategies to increase them such as early withdrawal of immunosuppression are warranted.

Table 1
Multivariable model for OS

Variable	Comparison	Hazard Ratio (95% CI)	p-value
CMV D90	R v NR	3.28 (1.19,9.05)	0.022
CD8 D90	CD8L v CD8H	3.87 (1.39,10.8)	0.0096
GVHD/Steroid D90	Y v N	0.71 (0.32,1.57)	0.40
Dz at HSCT	N v Y	0.27 (0.11,0.65)	0.0036
HCTCI	1 unit increase	1.43 (1.08,1.90)	0.013
Age	1 year increase	0.99 (0.97,1.02)	0.60
Conditioning	Myelo v RIC	1.36 (0.63,2.94)	0.44

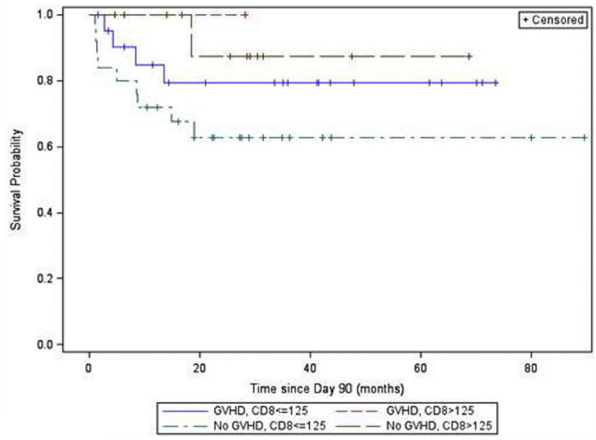

Figure 1.

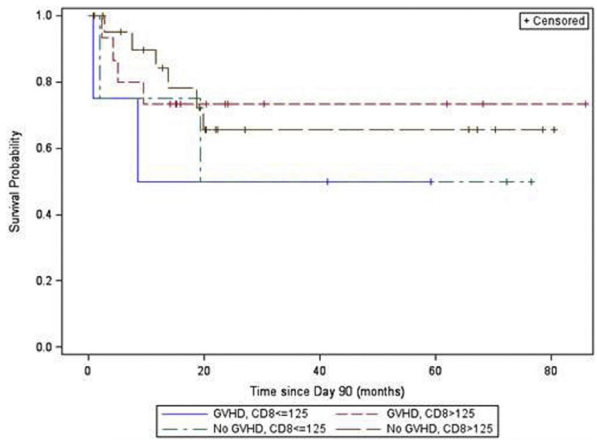

Figure 2.

205

Second Allogeneic Hematopoietic Cell Transplantation for Graft Failure: Poorer Outcomes for Neutropenic Graft Failure

Troy Christopher Lund [1], Jessica Liegel [2], Paul Orchard [3], Qing Cao [4], Jakub Tolar [2], Claudio Brunstein [5], John E. Wagner [6], Michael R. Verneris [7], Daniel J. Weisdorf [5]. *[1] Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, MN; [2] University of Minnesota, Minneapolis, MN; [3] Pediatrics Blood and Marrow Transplantation, University of Minnesota Masonic Cancer Research Building, Minneapolis, MN; [4] Biostatistics and Bioinformatics, University of Minnesota, Minneapolis, MN; [5] University of Minnesota Medical Center, Minneapolis, MN; [6] Pediatric Blood and Marrow Transplantation, University of Minnesota, Minneapolis, MN; [7] Pediatric Hematology and Oncology, University of Minnesota Medical Center, Fairview, Minneapolis, MN*

Graft failure (GF) after hematopoietic cell transplant (HCT) occurs in 5-30[DW1] % of patients. GF can be accompanied by neutropenia (NGF) or can result with adequate neutrophils, but loss of donor chimerism (non-neutropenic graft failure, NNGF). We analyzed the outcomes of 61 patients (pediatric and adult) treated with a second HCT for GF at the University of Minnesota; 27 with NGF and 34 with NNGF. The cumulative incidence of neutrophil engraftment at 42 days after second HCT was 88% for NNGF, and 68% for NGF ($p=0.03$). The incidence of grade III-IV acute graft versus host disease (GVHD) was 15% (95% confidence interval (CI), 2 – 28%) and 6% (95% CI, 2 - 17%) for NGF and NNGF, respectively ($p = 0.17$). From the 2ndHCT, 1-year