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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

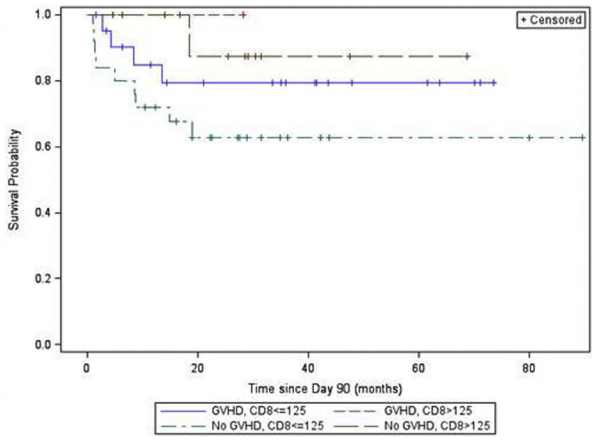

Figure 1.

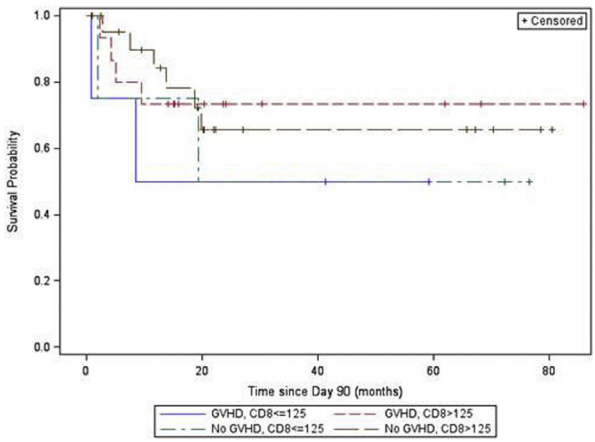

Figure 2.

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

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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

overall survival (OS) was 54% (95% CI, 41–66%) for the entire cohort with an OS for NNGF of 73% (95% CI, 55 – 85%) and for NGF OS was 30% (95% CI, 14–47%) ($p<0.01$). A second GF occurred in 18 (67%) NGF and in 9 (26%) NNGF patients. Viral studies showed there was more frequent HHV6 reactivation, with 48% of the NGF group versus 8.8% of the NNGF group ($p < 0.01$) having reactivation. EBV and CMV reactivate was not different. The most common cause of death after second HCT was persisting GF leading to infection or infection despite engraftment. Outcomes of second HCT for NGF and NNGF are different with very poor outcomes for the NGF group, necessitating new approaches to improve overall survival.

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Prolonged Stimulation-Induced Pro-Apoptotic B Cells and Deficits in the Germinal Center Formation of Memory B Cells within One Year after Allogeneic HSCT

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B-cell immune dysfunction substantially contributes to the risk of severe infections after allogeneic hematopoietic stem cell transplantation (alloHSCT). B-cell numbers normalize within one year after transplantation, however many patients display a slow recovery of $CD27^+$ memory B cells and week vaccination responses. Little is known about functional B-cell deficits associated with memory deficiency post-transplant.

In our study we quantitatively and phenotypically analyzed B- and T-cell subsets in peripheral blood by flow cytometry at days 180 and 360 after alloHSCT in acute leukemic patients (n=36). In addition, apoptosis of B-cell subsets was investigated after stimulation with CpG and CD40L. To address the B-cell milieu cytokines and chemokines were measured with Luminex technology.

Half of patients at day 180 and all patients at day 360 displayed fully restored absolute B-cell numbers, although $CD27^+$ memory B-cell subsets remained diminished (cells/μl±SEM: healthy control (HC) 26±4, alloHSCT 4±1; $p\leq0.001$). All B-cell subsets were characterized by an activated/pro-apoptotic phenotype with an increased CD86 and Fas but reduced Baff-R expression at day 180. Accordingly, an inflammatory milieu was present with increased serum levels of TNFa, IFNa2, G-CSF, IP-10, MCP-1, MIP-1b and Eotaxin in patients compared to HC. While CD86 normalized at day 360 on most B-cell subsets, all subsets retained a strong pro-apoptotic phenotype with increased Fas and reduced Baff-R expression. Interestingly, stimulation of peripheral blood mononuclear cells with CpG and CD40L resulted in significantly increased percentages of apoptotic cells for the patients compared to HC (% of B cells±SEM: HC 11±2, alloHSCT 28±5 day180, 60±6 day 360; $p\leq0.001$).

Regarding the lack of $CD27^+$ memory B-cell recovery, we assumed a defective germinal center (GC) reaction. Supporting this suggestion we found (A) a reduced CXCR5 expression on naïve B cells, important for the entry into B-cell follicles (MFI±SEM: HC 5899±975, alloHSCT 3494±554; $p\leq0.01$), (B) a low HLA-DR expression on naïve B cells, essential for antigen presentation (HC 20440±4742, alloHSCT 9150±2386; $p\leq0.01$), (C) a normal number of $CD27^-IgD^-$ double negative (DN) B cells, suggested to prematurely leave the GC reaction, (D) a remaining $CD4^+$ T-cell deficiency (cells/μl±SEM: HC 972±77, alloHSCT 303±45; $p\leq0.001$) and (E) a reduced percentage of circulating $CXCR5^+$ follicular T helper cells, importantly involved in GC reactions (%±SEM: HC 6±2, alloHSCT 2±1).

We conclude that a sustained inflammatory milieu and possibly damage within lymphoid organs might contribute to a prolonged pro-apoptotic state of B-cell subsets and defects in the GC reaction long-term after allo-HSCT. Despite normal total B-cell numbers significant functional deficits exist more than one year after transplant, so that adoptive transfer of memory $CD27^+$ B cells should be pursued.

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Immune Recovery (IR) Following Allogeneic Stem Cell Transplant (Allo-SCT): A Comparison of Three Different Transplant Strategies at a Single Transplant Center

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Introduction: Favorable IR after allo-SCT has been reported after reduced intensity conditioning (RIC); however, T-cell depletion and cord blood (CB) SCT have been associated with delay in IR. The goal of this study is to compare the rates of IR using three different transplant strategies employed at UMass Memorial Medical Center.

Patients & Methods: We retrospectively analyzed data of all patients who underwent allo-SCT at our institution since April 2009 using either RIC with thiotepa/fludarabine/melphalan followed by post-transplant cylophosphamide (TFM/Cy arm), RIC with Fludarabine/Busulfanx2/antithymocyte globulin (FluBu2/ATG arm) or CB transplant with TFM/ATG regimen. IR was assessed by rates of recovery of lymphocyte subsets (CD3, CD4, CD19, CD25+127- and NK-cells) and serum immunoglobulins (Ig's) at D30, D100 and 1 year post transplant.

Results: 102 patients were identified from the database. 38 patients (37.2%) were included in the TFM/Cy arm, 38 patients (37.2%) in the FluBu2/ATG arm and 26 patients (25.5%) in the CB arm. Median age of all patients was 62.2 years (range 18.4 – 83.5) and 52.4, 67.5, and 63 years in the TFM/Cy, FluBu2/ATG and CB arms, respectively. Median lymphocyte subset counts and Ig levels at different post transplant points are detailed in Table 1. CD3 and CD4 recovery was significantly inferior in the CB arm at D30 and D100. There was a trend towards delayed IR of regulatory T-cells (CD25+127-) in the CB arm at all points. CD19 and NK-cell recovery was superior in the CB arm at all points, but NK-cell recovery did not reach statistical significance at 1 year. Recovery of serum Ig's was noted to be faster in the FluBu2/ATG arm early on post transplant. At 1-year post allo-SCT, Ig values were comparable in all arms.