Inflammatory cytokines are thought to play a significant role in the toxicity of allogeneic BMT. We examined the correlation of plasma levels of TNF-α and outcomes in 64 patients receiving myeloablative allogeneic BMT using soluble TNF-α receptor 1 (TNFR1) as a surrogate marker for TNF-α. Because baseline values of TNFR1 pre-BMT ranged fifty-fold between patients (105-5876 pg/ml), all TNFR1 values post BMT were normalized to pre-BMT baseline. We first evaluated the relationship between TNFR1 and GVHD. Of the 64 subjects, 36 (56%) subjects had no GVHD and 28 (44%) had GVHD as their only serious post-transplant complication (mean day of onset, 28 d). On day 7, when no subject had GVHD, the mean TNFR1 ratio in subjects who remained GVHD-free was 2.2 compared to 4.6 for those who eventually developed GVHD (P = .003). Subjects were divided equally into low, medium and high categories of day 7 TNFR1 ratios and had rates of eventual GVHD of 27%, 33%, and 62%, respectively (P = .02). Seven subjects died before day 100, all from GVHD. Day 28 TNFR1 ratios (the mean day of onset of GVHD) correlated with mortality by day 100. The mean TNFR1 ratio on day 28 in subjects who survived to day 100 was 1.0 compared to a mean ratio of 10.4 in subjects who died before day 100 (P < .001). Subjects were divided equally into low, medium and high categories of day 28 TNFR1 ratios and had rates of death by day 100 of 8%, 20%, and 29%, respectively (P = .04). Of the 28 patients who developed acute GVHD and required treatment of the disease, 14 (50%) responded completely to high-dose steroids and 14 had incomplete responses. In patients with complete responses TNFR1 ratios decreased, and 8 weeks later were equivalent to ratios in patients never experiencing GVHD. By contrast, patients who did not completely respond to high-dose steroids showed increasingly elevated TNFR1 ratios that were significantly different at 8 weeks post-onset (P = .03). We conclude that post-myeloablative transplant TNFR1 ratios correlate with the subsequent development of GVHD and day 100 mortality. Subjects who develop GVHD who respond to treatment show falling TNFR1 levels while non-responders show increasing levels. We have expanded this preliminary study to an additional 150 patients with results pending. Further exploration of the predictive value of post-transplant TNFR1 levels in not completely responding patients may help predict complications days to weeks in advance of their clinical presentation.

139 CYCLOSPORIN A AND MINI SHORT-TERM METHOTREXATE VERSUS CYCLOSPORIN A AS GRAFT VERSUS HOST DISEASE PROPHYLAXIS IN PATIENTS WITH BETA THALASSEMA MAJOR UNDERGOING ALLOGENEIC BLOOD AND MARROW TRANSPLANTATION

Irrazani, M., Mousavi, A., Gholidehian, S., Bahar, B., Samiei, S., Abhari, A., Eghbal, L., Gharamezadeh, A. Hematology-Onology & Bone Marrow Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

In this study, we compared the effects of cyclosporine A (CsA) alone at graft-vs.-host disease (GvHD) prophylaxis versus cyclosporine with short course methotrexate (MTX) in patients with thalassemia who received hematopoietic stem cell transplantation from HLA-identical siblings. One hundred forty patients were enrolled in this study. The first group, 50 patients (class I and II), received CsA alone 3 mg/kg IV from day -2 to +5 followed by 12.5 mg/kg/day which was tapered according to the patient’s condition. The other group of 90 (class I, II, III) patients received the combination of CsA + MTX in which CsA was used with the above mentioned dose and MTX was 10 mg/m2 on day +1 and 6 mg/m2 on days +3 and +6. Incidence of acute GvHD grade II-IV in the CsA group was 78% and in the CsA + MTX group was 52.2%, which was statistically significant (P < .001). There was no significant difference in the incidence of chronic GVHD between two groups. The mean time to neutrophil engraftment to 0.5 x 10^9/L was 14 and 23 days for CsA group and CsA + MTX group, respectively (P < .001). There were no significant differences for platelet recovery between the two groups. Graft failure in the CsA and CsA + MTX groups was seven (14%) and nine (10%) patients, respectively (P = .58). The 5 year overall survival (OS) and disease free survival (DFS) was 77% and 58% in the CsA group versus 85% and 80% in the CsA + MTX group, respectively. OS had no statistically significant differences between two groups (P = .56) but there were significant differences for DFS between the two groups (P = .05). This study shows that the CsA and short term MTX for a GVHD prophylaxis regimen can reduce the incidence of aGvHD grade II to IV and improve the probability for survival in patients with thalassemia major given allogeneic blood and marrow transplantation.

140 SIGNIFICANT INCREASE IN CORD BLOOD (CB) VS PERIPHERAL BLOOD (PB) ALLOANTIGEN SPECIFIC T-REGULATORY (T-Reg) (CD4+/CD25+/CTLA4+*) CELLS FOLLOWING ANTIGEN PRESENTING CELL (APC) INDUCTION: MOLECULAR, FUNCTIONAL AND IMMUNOPHENOTYPIC CHARACTERIZATION

Cairo, M.S., Sarwani, P., Oberfield, N., Vlad, G., Simpson, L., van de Ven, C., Chang, C. Columbus University, New York, NY.

We have demonstrated that HLA disparate (2-3 antigen) CB can successfully be used as an alternative allogeneic (allo) cell source and more importantly, is associated with decreased severe acute and chronic GVHD (Cairo et al., Blood, 1997). Studies from our lab have suggested an immaturity in CB T cell immunity in part secondary to decreased IL-12, IL-15 and IL-18 gene expression and protein production. However, CB T reg cells may also actively and specifically suppress unwanted allo immune responses. In this study we compared the functional and molecular events with the induction of allo specific CB vs PB T-reg cells. Briefly, Mutz-3 immature dendritic cells (iDC) and monoocyte derived (GM-CSF/IL-4/IL-10) (m)DC were utilized for APC in vitro stimulation. CD4+ CD4+/CD25+ and CD4+/CD25+ CB and PB were purified by CD4 and CD25 selection (Miltenyi, Auburn, CA). CD4+/CD25+ proliferation was determined by in vitro priming with iDC or mDC 1:5 ratio with 3H-thymidine in both primary and secondary MLR reactions. T-reg suppression was measured with CD4+CD25+ iDC at a 1:1.101 ratio. RT-PCR was used to measure gene expression of GITR, FoxP3, CTLA-4, CD25, GAPDH and flow cytometry for GITR, CTLA-4, CD25 and IL-10 expression. Naive PB CD4+/CD25+ (T-reg) significantly suppressed CD4+/CD25+ proliferation compared to CB T-reg (60% vs 10%, P < .02). However, in the secondary MLR response CB vs PB CD4+ proliferation was significantly less with Mutz-iDC (10.5 ± 3.3 vs 48.5 ± 13.1 x 10^3 cpn, P < .02) suggesting a decrease in allo response of CB vs PB CD4+ T cells. Naive CB vs PB CD4+/CD25+ T-reg had similar gene expression of GITR, FoxP3, -4 and CD25. However, GITR protein expression was significantly higher in CB vs PB (11 ± 3 vs 3.4 ± 1.6%, P < .05). Following Mutz-iDC induction of CD4+/CD25+, T-reg cells demonstrated similar gene expression of CTLA-4, GITR, FoxP3 and CD25 (3-8 fold) with CB vs PB; however, there was a significant increase in CB vs PB protein expression of CD25 (50 ± 1 vs 35 ± 2%, P < .02) and CTLA-4 (25.2 ± 5 vs 12.5 ± 0.9%, P < .05). Furthermore, CB T-reg suppressive activity was allo-antigen specific (Mutz-iDC vs MHC I mismatched allo-mDC: 31.5 ± 4 vs 73 ± 10.5%, P < .001) and IL-10 independent. In summary, there is a significant increase in allogeneic specific APC induction of CB vs PB CD4+/CD25+ T-reg cells which may in part be responsible for the suppression and decrease in alloantigen recognition and immune response of CB CD4+/CD25+ T cells.