Severe, acute graft versus host disease (aGvHD) contributes significantly to morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Diagnosis of GvHD is mainly based on clinical features and tissue biopsies, a non invasive, unbiased laboratory test for GvHD diagnosis does not exist. To establish such a test, capillary electrophoresis coupled online with mass spectrometry (CE-MS) was applied to a training set consisting of 13 urine samples from 10 allo-HSCT patients with aGvHD ≥ grade II and 50 urine samples of 23 patients without GvHD.

Thirty one potential GvHD-specific polypeptides were chosen out of 300 differentially excreted peptides, based on the high discriminatory values allowing differentiation of patients with aGvHD from those with no GvHD. This pattern was then used to characterize urine samples from 141 patients that were collected prospectively and blinded. The aGvHD specific model allowed correct classification of 13/13 (sensitivity 100 % [95 % CI 75.1 to 100.0]) aGvHD samples and 49/50 (specificity 98.0 % [95 % CI 89.3 to 99.7]) control samples of the training set. The subsequent blinded evaluation on 599 samples collected from 141 patients after allogeneic HSCT enabled the diagnosis of aGvHD ≥ grade II up to 14 days prior to clinical symptoms with a sensitivity of 81.4 % [95 % CI 73.1 to 87.9] and a specificity of 75.7 % [95 % CI 71.6 to 79.4].

High resolution proteome analysis may help to identify patients with aGvHD in an unbiased laboratory based screening, possibly enabling pre-emptive therapy.

**39 DIFFERENTIAL IMPACT OF mTOR INHIBITION ON FOXP3+ REGULATORY T CELLS AS COMPARED TO CONVENTIONAL T CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION**

Zeiser, R.1, Lesecon-Gower, D.B.2, Zambriksi, E.A.1, Hon, J.-Z.2, Negrin, R.1, Stanford University School of Medicine, Stanford, CA.

FoxP3+CD4+CD25+ regulatory T-cells (Treg) have been shown to effectively reduce the severity of experimental acute graft-versus-host disease (aGvHD) while sparing graft-versus-leukemia activity. These findings, in concert with the observation that human and murine Treg share functional characteristics, have fueled interest in clinical trials to control aGvHD. Recent data indicates that the immunosuppressant rapamycin (RAPA) in contrast to cyclosporine A does not interfere with in vivo function of Treg, but instead can enhance Treg expansion in vitro by a yet unknown mechanism. To investigate the impact of mTOR inhibition on proliferating Treg and Tconv, both cell types were stimulated in the presence of different RAPA concentrations in vitro. Phosphorylation of mTOR downstream products p70S6K1 and 4E-BP1 were assessed by western blot and flow cytometry. Inhibition of the phosphorylation of p70S6K1 and 4E-BP1 was observed in both populations in the presence of RAPA. Interestingly, Treg were more resistant to mTOR inhibition as compared to Tconv and displayed significantly higher phosphorylated products in the presence of RAPA at 10 nM (MFI Treg vs Tconv, p<0.001) and at 100nM (MFI Treg vs Tconv, p<0.001). To investigate whether Treg and RAPA protect from aGvHD in a synergistic manner, BALB/c recipients were transplanted with H-2 disparate BM and 1.6X10^6 T-cells (FVB/N) after lethal irradiation. aGvHD lethality was only slightly reduced when suboptimal Tconv:Treg ratios were employed, or when recipients were treated with a non-protective RAPA dose (0.5 mg/kg bodyweight). Combining a suboptimal Tconv:Treg ratio with a non-protective RAPA dose reduced expression of luciferase expressing (Luc+) Tconv and pro-inflammatory cytokines and improved survival indicative for an additive in vivo effect of RAPA and Treg. To evaluate the impact of RAPA on in vivo T cell expansion, either Luc+ Tconv or Luc+ Treg were adaptively transferred. In vivo bioluminescence imaging demonstrated that RAPA had a more potent inhibitory effect on proliferation of Tconv as compared to Treg (p<0.05 vs. NS). We did not observe RAPA to increase FoxP3+ Treg numbers in vivo. Thus, increased Treg numbers observed in RAPA containing expansion cultures are likely due to a lower susceptibility of this cell population to mTOR inhibition. This could explain the observed synergistic effect of RAPA and Treg in aGvHD protection which has relevance for clinical trials utilizing Treg to prevent aGvHD.

**HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES**

**40 HUMAN LEUKOCYTE ANTIGEN (HLA) HAPLOTYPES AND SINGLE HLA ANTIGENS IN HLA-MATCHED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLOHST) FOR MYELOID DISEASES: ANALYSIS OF OVERALL AND PROGRESSION FREE SURVIVAL (OS/PFS)**

Aggarwal, C.1, Gupta, S.1, McCarthy, P.L.2, Battistella, M.2, Hahn, T.E.3 Department of Medicine, State University of New York at Buffalo, Buffalo, NY; 2Blood and Marrow Transplant Program, Roswell Park Cancer Institute (RPCI), Buffalo, NY.

Donor and recipient mismatching in the HLA system is a major factor influencing alloHSCT outcome. There is increasing evidence that specific HLA antigens influence immunobiological outcomes even in HLA-matched alloHSCT. This could be related to the physiological role of the HLA system in differential antigen presentation. In contrast, HLA haplotypes are associated with multiple immunopathologic diseases by possible immunoregulatory response modulation in an antigen-independent manner. We recently reported an association of HLA haplotypes with acute GvHD. We present results of a retrospective review of 119 related and 48 unrelated consecutive first alloHSCT pts treated at RPCI from 1992 to 2003 to investigate the association of the 3 most common haplotypes in Caucasians (A1B8DR3, A3B7DR15 and A2B44DR4) and HLA antigens, in addition to standard transplant risk factors, on OS and PFS. HLA typing was determined by either molecular (n=108) or serologic (n=9) methods. All analyzed patients were matched at HLA-A, -B and -DRB1 antigen level. Pt characteristics included: 50% AML, 38% CML and 12% MDS; median age 43 years (range 11-66), 60% >40 years; 62% Male; >95% Caucasian; 73% Recipient-Donor Sex Match; 78% TBI-containing conditioning regimens; and 88% bone marrow stem cell source. There were no significant differences in pt characteristics by the presence or absence of each haplotype or antigen. By univariate analysis, HLA-A24 (p=0.047) was significantly associated with improved OS whereas HLA-B8 (p=0.034), KPS <80 (p=0.0045), >40 yrs (p=0.0014), and TBI-containing regimens (p=0.016) were associated with decreased OS. HLA haplotype A1B8DR3 trended towards inferior OS (p=0.09). Multivariate analysis found that HLA-B8, age >40 yrs, KPS <80 and TBI-containing regimens were significant independent predictors of decreased OS. Univariate analysis showed that HLA-B8 (p=0.0003), -DR3 (p=0.026) and A1B8DR3 (p=0.028) were significantly associated with inferior PFS. Multivariate analysis found HLA-B8 was the only significant factor associated with decreased PFS. In Caucasians, HLA-B8 is rarely seen outside the A1B8DR3 haplotype, therefore it is not possible to distinguish differential antigen presentation from non-specific inflammation as underlying immunobiologic mechanisms for the survival differences. Larger patient population analyses are required to differentiate these mechanisms.