Historically, the most important adverse risk factors for survival after BMT for AML are Active disease (ActDis), poor-risk cytogenetics, and older age. High serum LDH at the time of diagnosis is an adverse risk factor for survival and AML in the absence of BMT, but has not been explored as a risk factor in setting of BMT. We performed a multivariable analysis of these and other potential risk factors for overall and progression-free survival. From August 1992 to July 2005, we treated 87 patients with AML, who also had informative cytogenetic studies from the time of diagnosis, with high-dose busulfan-containing preparative regimens and an HLA-matched sibling BMT. The median age was 43 years (range 19 to 62). The median LDH level at the time of BMT was 204 U/L (range 93-1555 U/L; normal 100-220 U/L). Forty-one patients were in either first (n = 30) or second complete remission (n = 11; CR). 46 patients were with ActDis were treated. The 87 patients were then classified according to the SWOG/ECOG (Blood 96: 4075, 2000), MRC (Blood 92:2322, 1998), and CALGB (Blood 100:4325, 2002) cytogenetic classification systems. With a median follow-up of 56.0 months (range 4.5-107.8 months), the median progression-free survival is 13.5 months for patients in CR1 and 4.1 months for patients in CR2. The progression-free survival of patients with ActDis was 5.5 months. A Cox proportional hazards analysis that included gender, age, LDH, disease status, all three cytogenetic risk groups, preparative regimen, source of stem cells, and CMV status was performed for overall and progression-free survival. Significant risk factors for shorter survival in univariate analysis included male gender, LDH >330 U/L (but not LDH >220 U/L or per 100 U/L increase), and peripheral stem cells as a source of hematopoietic reconstitution (n = 7). Surprisingly, a male donor to a male recipient was also an adverse risk factor (p<0.001). However, in multivariable analysis, only LDH >330 U/L (p=0.002), source of stem cells (p=0.019), and male donor to male recipient (p<0.001) remained as significant adverse risk factors. In fact, patients with ActDis and an LDH > 330 U/L had similar survival to patients treated in remission, while patients with ActDis and LDH > 330 U/L had significantly worse survival. We conclude that high LDH (>1.5X upper limit of normal) at the time of BMT is a significant adverse risk factor for survival after BMT for AML.

**NOD2/CARD15 GENE SINGLE NUCLEOTIDE POLYMORPHISMS ARE ASSOCIATED WITH SIGNIFICANT INCREASES IN MORTALITY AND TO INCREASES IN DISEASE RELAPSE IN RECIPIENTS OF AN UNRELATED DONOR HAELOMATOPOIETIC STEM CELL TRANSPLANT FOR ACUTE LEUKAEMIA**

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SNPs of the Nod2/CARD15 Gene, a candidate gene for susceptibility to inflammatory disorders, have been associated with disease progression and mortality in other hematopoietic disorders and with disease relapse and mortality in Acute Leukemia (HL) and Non-Hodgkin’s lymphoma (NHL). However, the impact of NOD2/CARD15 SNPs on outcome after allogeneic hematopoietic stem cell transplantation (HSCT) has not been explored.

61 Forty-eight (83%) patients had received a prior autologous (auto) SCT. The median time to progression after auto-SCT was five months (1-34). Disease status at SCT was sensitive relapse (n = 30) or refractory relapse (n = 28). The conditioning regimen employed was fludarabine (125-130 mg/m sq over 4-5 days), melphalan (140 mg/m sq IV over 2 days) (FM) and antithymocyte globulin (thymoglobulin 6 mg/kg over 3 days) was added for the most recent fourteen MUD transplants. Chimisumms included 100% donor-derived engraftment in all patients (100%). Cumulative 100-day and 2-year transplant-related mortality (TRM) were 7% and 15%, respectively, (100-day TRM MRD < MUD 6% vs. 8%, p=0.8; 2-year MRD vs. MUD 13% vs. 16%, p=ns). The cumulative incidence of acute (grade II-IV) GVHD (first 100 days) was 28% (MRD vs. MUD 12% vs. 39%, p=0.04). The cumulative incidence of chronic GVHD at any time was 74% (MRD vs. MUD 57% vs. 89%, p=0.003). Fourteen pts (24%) received a total of 25 (range 1-5) donor leukocyte infusions (DLIs) for disease progression/relapse (PD). Five of them (35%) received chemotherapy as well, and none (64%) developed acute GVHD after the DLI. Thirty-six patients (62%) are alive (23 in remission) with a median follow-up of 24 months (4-78). The f/up is 23 months (4-53) for alive pts always in remission. Twenty-two patients (38%) expired, and relapse-related mortality was 24%. Projected 2-year overall (OS) and progression-free (PFS) survival are 64% (49-76) and 32% (20-45), with 2-year projected PD at 55% (41-70). There was no statistically significant difference between MRD and MUD transplants with regard to OS (p=0.1), PFS (p=0.9) and PD (p=0.8). There was a trend for the response status prior to allo-SCT: complete response (15% of conditioned patients, complete response unde- fined vs. all others) to favorably impact PFS (p=0.07) and PD (p=0.049), but not OS (p=0.4). Partial responders and patients

81% using bone marrow. Two forms of post-transplant immuno- suppression predominated, Cyclosporine A and Methotrexate (47%) and Cyclosporine A alone (81%).

SNPs of the NOD2/CARD15 gene within an UD-HSCT pair resulted in a significant increase in disease relapse and mortality. The estimated two-year incidence of disease relapse was 43% in wild type pairs as compared to 70% in pairs with NOD2/CARD15 SNPs (Log rank, P=0.0008). The estimated three-year overall survival was 22% and 44% for pairs with and without NOD2/CARD15 polymorphisms respectively (Log rank, P=0.0087). These findings persisted in multivariable analysis. NOD2/CARD15 genotype was found to be the most significant factor for an adverse outcome other than being transplanted in a disease stage of relapse.

In contrast to previous findings, the presence of NOD2/CARD15 SNPs in an Acute Leukaemia UD-HSCT pair results in a significant increase in disease relapse and subsequently a decrease in mortality. These novel data show an important role for NOD2/CARD15 genotyping in transplantation and suggest a possible effect of the NOD2 protein in alloreactivity and tumour surveil- lance. Genotyping recipients and donors prior to transplant may allow for prediction of transplant outcome, and thus present crit- ical information for donor selection.

**LYMPHOMA/MULTIPLE MYELOMA**

62 **TWO-YEAR FOLLOW-UP RESULTS AT THE M.D. ANDERSON HOSPITAL WITH REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE-MELPHALAN AS PREPARATIVE REGIMEN IN RELAPSED/REFRACTORY HODGKIN’S LYMPHOMA**

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Fifty-eight patients with relapsed or refractory Hodgkin’s lymphoma (HL) underwent allogeneic stem cell transplantation allo-SCT following a reduced-intensity conditioning (RIC) regimen from a matched related donor (MRD; n=25) or a matched unrelated donor (MUD; n=33). The median age was 32 years (range 19-59). Forty-eight (83%) patients had received a prior autologous (auto) SCT. The median time to progression after auto-SCT was five months (1-34). Disease status at SCT was sensitive relapse (n=30) or refractory relapse (n=28). The conditioning regimen employed was fludarabine (125-130 mg/m sq over 4-5 days), melphalan (140 mg/m sq IV over 2 days) (FM) and antithymocyte globulin (thymoglobulin 6 mg/kg over 3 days) was added for the most recent fourteen MUD transplants. Chimisumms indicated 100% donor-derived engraftment in all patients (100%). Cumulative 100-day and 2-year transplant-related mortality (TRM) were 7% and 15%, respectively, (100-day TRM MRD < MUD 6% vs. 8%, p=0.8; 2-year MRD vs. MUD 13% vs. 16%, p=ns). The cumulative incidence of acute (grade II-IV) GVHD (first 100 days) was 28% (MRD vs. MUD 12% vs. 39%, p=0.04). The cumulative incidence of chronic GVHD at any time was 74% (MRD vs. MUD 57% vs. 89%, p=0.003). Fourteen pts (24%) received a total of 25 (range 1-5) donor leukocyte infusions (DLIs) for disease progression/relapse (PD). Five of them (35%) received chemotherapy as well, and none (64%) developed acute GVHD after the DLI. Thirty-six patients (62%) are alive (23 in remission) with a median follow-up of 24 months (4-78). The f/up is 23 months (4-53) for alive pts always in remission. Twenty-two patients (38%) expired, and relapse-related mortality was 24%. Projected 2-year overall (OS) and progression-free (PFS) survival are 64% (49-76) and 32% (20-45), with 2-year projected PD at 55% (41-70). There was no statistically significant difference between MRD and MUD transplants with regard to OS (p=0.1), PFS (p=0.9) and PD (p=0.8). There was a trend for the response status prior to allo-SCT: complete response (15% of conditioned patients, complete response unde- fined vs. all others) to favorably impact PFS (p=0.07) and PD (p=0.049), but not OS (p=0.4). Partial responders and patients
with stable/refractory disease fared similarly with regard to OS and PFS. Day 100, 2-year TRM and OS/PFS data appear very encouraging in these very high-risk, extensively pretreated patients. Response status at transplant seems to affect outcome, and PD remains a major obstacle.

**63 VACCINATION WITH DENDRITIC CELL MYELOMA FUSIONS ALONE OR IN CONJUNCTION WITH STEM CELL TRANPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA**

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We have demonstrated that patient derived myeloma cells fused with autologous dendritic cells (DCs) potently stimulate anti-tumor immunity in vitro. We are conducting phase I clinical trials in which patients with myeloma undergo serial vaccination with DC/myeloma fusions alone or in conjunction with stem cell transplantation. To date, 18 patients have been enrolled (11-vaccine alone, 7 vaccine following autologous stem cell transplant). To generate mature DCs, adherent mononuclear cells were isolated on a lymphocyte separation collection and cultured with GM-CSF, IL-4, and TNFα. The mean yield and viability of the DC preparations was 1.5 x 10^6 cells and 88%, respectively. Patient derived myeloma cells were isolated from bone marrow aspirates and were quantified by the expression of CD38 and/or CD138. The mean yield and viability of the myeloma cell collections was 7.3 x 10^6 cells and 89%, respectively. Fusion cells were generated by coculture of DCs with myeloma cells in the presence of 50% polyethylene glycol. The mean fusion efficiency was 40% as determined by the percentage of cells that co-expressed unique DC and myeloma antigens. In contrast to myeloma cells, the DC and fusion cell preparations prominently stimulated allogeneic T cell proliferation in vitro. To date, 13 patients have completed vaccination at a dose of 1.5 x 10^6 fusion cells, GM-CSF (100 µg) was administered subcutaneously on the day of vaccination and for 3 days thereafter. Adverse events judged to be potentially vaccine related have included vaccine injection site reactions, edema, rash, fever, chills, fatigue, muscle aches, pruritis, and diarrhea. One patient with a history of deep venous thrombosis (DVT) developed a DVT and pulmonary embolus of uncertain relation to the vaccine. To date, a majority of evaluable patients demonstrated evidence of vaccine induced anti-myeloma immunity as demonstrated by at least 2 fold increase in IFNγ expression by CD4 and/or CD8 T cells in response to ex vivo exposure to autologous tumor lysate. Of patients undergoing vaccine therapy alone, 5 patients demonstrated stabilization of the myeloma paraprotein for 2-6 months following initiation of vaccination. Of 3 patients completing post-transplant vaccination, 2 patients demonstrated resolution of the persisting myeloma protein post-transplant and 1 patient demonstrated a transient increase followed by a decline in paraprotein levels post-transplant.

**64 INTERLEUKIN-6 AND HEMATOPOIETIC SOLUBLE FACTORS CONFER RESISTANCE TO TRAIL MEDIATED APOPTOSIS**

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The bone marrow microenvironment provides tumor protection from chemotherapy and Fas-death receptor mediated apoptosis through a process known as environmental mediated death resistance (EM-DR). TRAIL, a member of the TNF superfamily, has anti-tumor activity sparing normal cells and mediates allogeneic graft-versus-tumor responses. We hypothesized that the tumor microenvironment influences TRAIL mediated killing conferring an immune resistance mechanism contributing to cancer progression in allogeneic transplantation. Experiments in a transwell (TW) assay with RPMI-8226/Myeloma cells in the upper well and HS5 hematopoietic stromal cells in the bottom well (TW+HS5) revealed that HS5 cells blocked recombinant human (rh)-TRAIL-induced apoptosis through soluble factors in a dose and time-dependent manner. RPMI-8226 treated in TW+HS5 exhibited attenuated pro-caspase-8, pro-caspase-3, PARP, and BID cleavage, with diminished mitochondrial membrane potential changes, without alterations on TRAIL receptors. Western blotting of RPMI-8226 cells showed that Fas-associated death domain like IL-1 converting enzyme-like inhibitory protein (FLIP), a regulatory factor that competes with caspase-8 inhibiting apoptosis, is increased in TW+HS5 treated cells. Subcellular fractionation of RPMI-8226 cells showed that FLIP is maintained in or associated with organelle membranes and is released to the cytosol when exposed to soluble factor (TW+HS5) suggesting that that soluble factor signaling may influence FLIP localization and availability. FLIP reduction by FLIP-α RNA interference or with Bortezomib, NF-KB inhibitor that reduces FLIP levels, increases TRAIL apoptosis sensitivity of RPMI-8226 cells treated in TW+HS5. To explore whether IL-6, important myeloma survival factor, is involved in TRAIL-EMDR, RPMI-8226 treated with HS27a stroma that lack IL-6 secretion elicit a diminished apoptosis resistance compared to HS-5. In addition, RPMI-8226 treated in TW+HS5 with an anti-human IL-6 neutralizing antibody partially overcome the TRAIL apoptosis resistance. Furthermore, rh-IL-6 confers resistance to TRAIL mediated apoptosis in a dose-dependent manner associated with FLIP increase. Our results suggest that IL-6 and other soluble factors produced by marrow stromal cells promote myeloma cell survival by upregulating FLIP, thereby mitigating the influence of the microenvironment on TRAIL-induced apoptosis. The immune cytotoxic effect of TRAIL may be enhanced by FLIP inhibitors.

**65 THE USE OF CYCLOPHOSPHAMIDE (CY), FLUDARABINE AND ATG AS A PREPARATIVE REGIMEN FOR UNRELATED BLOOD TRANSPERNENT (UCBT) IN FANCONI ANEMIA: A SINGLE CENTER EXPERIENCE IN 27 PATIENTS**

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Stem cell transplantation from HLA identical siblings can cure over 80% of FA pts when they develop bone marrow failure syndromes. Unrelated CB is a good option for pts lacking a matched bone marrow unrelated donor, but the transplant procedure is complicated by a high incidence of graft failure and delayed immune recovery. From 01/03 to 05/06 we transplanted 27 pts with FA in aplastic phase. Gender: 8M/19F. Age: 4-19years (M:8y). Disease duration: 19-78months (M:36m). Previous treatments: 1-85UI (M:12). HLA Compatibility: 6/6:2pts; 5/6:9pts and 4/6:15pts. Preparatory regimen: CY60mg/kg; Fludarabine125mg/m2; MTX: 5pts. TNC infused before thawing: 2,3 – 19,2 x 10^6/kg. GVHD Prophylaxis: Cyclosporine (CsA) + steroids:22pts or CsA + MTX: 5pts. TNC infused before thawing: 2,3 – 19,2 x 10^6/kg (M: 5; 4,5). One pt received a double UCBT with a TNC of 5,2 x 10^7. Results: 11pts are alive between 113-1301 days after UCBT(M: 806d). 21pts survived > 28 and were evaluable for engraftment. 13pts (48%) had a complete hematological recovery and the median time to reach ANC>500/ul was 23 d(12-35d) and platelets > 20000/ul was 27 d(15-50d). Primary graft failure occurred in 6pts and 2pts had only a neutrophil engraftment. Most pts (6/8pts) without hematological recovery received a 4/6 CB. No pt survived a 2nd or 3rd UCBT. Post-transplant complications: Mucositis grade III-IV: 10pts (grade IV: 1pt). Hemorrhagic cystitis: 5pts. Hemolytic uremic syndrome: 1pt. Moderate/severe hypertension: 70% of pts. Neuro-