Human CD34+ cells were isolated from umbilical cord blood, stained with the membrane fluorescence marker PKH26, cultivated in cytokine rich media for five days and then sorted for their PKH26 fluorescence (dim versus bright, i.e. FDF versus SDF). Sorted cells were injected into the tail vein of sublethally irradiated NOD/SCID mice (2×10^5 cells/animal). The animals were sacrificed at defined time points from week 2 to 18 after transplantation. Flow cytometric analysis of the samples collected from the marrow, spleen, thymus, and lymph nodes of the NOD/SCID mice indicated that both SDF and FDF were able to engraft the bone marrow of the animals, i.e. erythroid and myeloid progenitor cells were recovered from the bone marrow of SDF and FDF repopulated mice. The most remarkable finding was that only cells of SDF origin were found in the lymph nodes and thymus where, at week 15 they differentiated into T cells. CD4+CD8+ double positive T progenitor cells were recovered in the thymus and CD4+ as well as CD8+ T cells were present in the spleen and the lymph nodes. Until 18 weeks after reconstitution, very few B cells were present in the spleen, whereas monocytes reached normal range. Global gene expression profiles of the two subpopulations (SDF and FDF) were analyzed using a human transcriptome cDNA microarray. Several molecular markers for stem cells were highly expressed in the SDF as compared to FDF: CD133 (Prominin), MDR1 (multiple drug resistance gene 1), clqr1 (complement component 1 receptor 1), Hoxa9, Cdx1 and Hesx1 (which encode homeodomain proteins).

Our data indicated that SDF was enriched for primitive human HSC, which were able to engraft in the thymus and lymph nodes in the SCID mouse model. These cells were able to give rise to precursor as well as mature T cells. Our results have provided unequivocal evidence that SDF is associated with primitive HSC function, and is consistent with the results of differential gene expression analysis in SDF. The significance of the cell-cell contacts in the SCID model in maintaining stemness is currently being examined.

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TARGETING MUC1 AS A MARKER FOR MYELOID LEUKEMIA STEM CELLS BY DC/AML FUSIONS

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The epithelial mucin antigen (MUC1) is aberrantly expressed in many epithelial tumors and hematologic malignancies and has served as a target for cellular immunotherapy. In this study, we examined MUC1 as a marker for myeloid leukemia cells and their progenitors. Myeloid leukemia cells were isolated from bone marrow aspirates or peripheral blood. MUC1 was not expressed on unselected leukemia cells (mean expression 3%, n = 12). A subset of samples underwent CD34 selection by magnetic bead separation. In contrast to unselected cells, 38% of CD34+ leukemia cells expressed MUC1 (n = 5). The leukemia stem cell compartment was isolated by separating CD34+/CD38-/lineage- fractions by flow cytometric sorting. Leukemia stem cells expressed MUC1 both by immunohistochemistry and FACS analysis. Similarly, we examined MUC1 expression on progenitor cells derived from chronic phase CML and following blast transformation. MUC1 was seen in only 4% of CD34+ cells obtained from chronic phase CML samples (n = 4) while uniform expression was observed in samples derived from patients with accelerated/blast phase. These data suggest that MUC1 serves as a marker for early leukemia progenitors and is associated with blastic transformation. We assessed the capacity of a dendritic cell (DC)/myeloid leukemia fusion cell vaccine to stimulate immune responses that target MUC1 and other antigens expressed by the stem cell compartment. DCs were generated from adherent mononuclear cells cultured with GM-CS, IL-4 and TNFa. DCs were fused with patient derived myeloid leukemia cells using polyethylene glycol. Fusion cells were quantified by determining the percentage of cells that expressed unique DC and leukemia antigens. Stimulation of autologous T cells with DC/AML fusions resulted in a mean 3 fold increase in CD8+ cells binding the MUC1 tetramer (N = 4). DC/AML fusions stimulated anti-tumor immune responses that targeted leukemia stem cells. Fusion stimulated T cells demonstrated increased expression of IFNγ following exposure to lysate generated from unselected leukemia cells (29 fold) and leukemia stem cells (28 fold). In contrast, exposure to renal carcinoma lysate generated only a 5 fold increase in IFNγ. In summary, this data suggests that leukemic progenitors in AML and accelerated/blast phase CML express MUC1. DC/tumor fusion vaccines target MUC1 and the stem cell compartment, and may be a potent immunotherapeutic strategy to eliminate the malignant stem cell clone in AML.

SUPPORTIVE CARE

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PREVENTION OF LATE CMV DISEASE AFTER HCT: A RANDOMIZED DOUBLE-BLIND MULTICENTER TRIAL OF VALGANCICLOVIR (VGCV) PROPHYLAXIS VERSUS PCR-GUIDED GCV/VGCV PREEMPTIVE THERAPY

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Background: Late CMV disease is an important complication after allogeneic HCT. PCR surveillance followed by preemptive therapy (PET) is costly and often not feasible. VGCV prophylaxis may prevent late CMV-related complications. **Methods:** Between day 80 and day 120 after HCT, CMV seropositive or D+/R- allograft recipients with CMV infection prior to day 80 or GVHD were randomized to receive VGCV prophylaxis (900 mg/day) or placebo (Plac) until day 270. Patients were monitored weekly for CMV DNA by plasma PCR, blood chemistry and CBC/differential. IV GCV (5 mg/kg twice daily) or VGCV (900 mg/kg twice daily) was given if CMV DNA was > 1000 copies/mL. The primary endpoint was a composite of CMV disease or invasive bacterial/fungal infection or death by day 270 and the study was designed to show superiority of VGCV prophylaxis. Follow-up was until day 640 after HCT. Neutropenia was analyzed while receiving study drug and by day 270. All analyses were by intent-to-treat. **Results:** 184 patients were randomized. All patients completed follow-up through day 270; 84% (VGCV) and 82% (Plac) completed follow-up through day 640 (as of 9/30/2007). There was no observed difference between the groups in the primary endpoint at day 270 (VGCV: 20%, Plac: 21%, hazard ratio [HR] 1.0, 95% CI 0.5–1.9, P = 0.96) and 640 (39% and 40%, HR 0.9, 95% CI 0.6–1.5, P = 0.8). Valganciclovir prophylaxis reduced CMV PCR positivity (11% and 36%, HR 0.3, 95% 01.-0.5, P = 0.0002). There was a trend towards more neutropenia on double-blind study drug (absolute neutrophil count [ANC] < 1000/mm^3 43% and 29%, HR 1.7, 95% CI 0.9–2.9, P = 0.08) but no significant difference was observed at day 270 (HR 1.3, 95% CI 0.8–2.1, p = 0.21 for ANC < 1000/mm^3). The number and severity of adverse events AEs (including SAEs) was similar between the groups. There was also no significant difference in secondary endpoints, including CMV disease at day 270 (2% in both groups, HR 0.9, 95% CI 0.1–6.5) and 640 (6% and 5%, HR 1.2, 95% CI 0.3–4.5), HSV/VZV infections by day 640, death at any time, or relapse of the underlying disease. **Conclusion:** VGCV prophylaxis was not superior in reducing the composite endpoint of CMV disease, invasive bacterial or fungal infection or death. The incidence of CMV disease was low in both groups. VGCV reduced CMV DNAemia and the need for PET. The rate of neutropenia and other AEs was high but not significantly different between the groups. The study was not designed to test for equivalency.

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AN INTERNATIONAL COMPARISON OF CURRENT STRATEGIES TO PREVENT HERPESVIRUS AND FUNGAL DISEASES IN HCT RECIPIENTS

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Background: Little is known about the prevention practices used by HCT centers internationally to prevent herpesvirus and fungal infections. The purpose of this survey was to compare prevention strategies by geographic region, center size and patient population (pediatric vs. adult). **Methods:** A web-based questionnaire was distributed to Program Directors at CIBMTR-affiliated HCT centers to elicit information on the strategies used to prevent HSV, VZV, CMV and fungal diseases at each institution between 1999 and 2003. **Results:** The response rate was 80% (n = 174) from HCT centers in 32 countries, including the US/Canada (n = 96), Europe (n = 40), Australia and New Zealand (n = 13), Latin America (n = 12), Asia (n = 6), the Middle East (n = 4) and South Africa (n = 3). While short-course acyclovir (ACV) for HSV prophylaxis was used almost uniformly, 62% of centers routinely used prophylactic low-dose ACV or similar agents as prophylaxis against VZV; of these, 19% treated for 1 month or less, 29% for 3–4 mos., 9% for 6–9 mos., 26% for 12 mos. and 17% until off immunosuppressants or until immune reconstitution. Strategies to prevent CMV disease in seropositive recipients were used in the following proportions: high-dose ACV/VACV alone (3%), Ganciclovir-based (GCV) prophylaxis alone (5%), surveillance and preemptive therapy (PET) alone (47%) or a combination strategy (45%) most commonly high-dose ACV and PET. The prevention strategies used in Europe were very similar to those used in the US with the exception of high-dose ACV/VACV which was used routinely by 48% of European centers compared with 25% of US centers ($P < .05$, Fisher exact test). In Australia and NZ (grouped together), GCV prophylaxis was used in higher proportions than in the US, Europe and Canada (54% vs. 23%, $P < .05$). Routine antifungal prophylaxis was used by 78% and 57% of European and Canadian centers, respectively, compared with its use in the US (97%), Australia/NZ (100%), and Latin America (100%). Also, antifungal prophylaxis was more commonly used in pediatric centers than in adult-only centers (97% vs 85%). Practices among smaller centers were remarkably similar to those in high-volume centers (>50 HCTs annually). Other trends in prevention strategies will be presented at the conference. **Conclusion:** This large analysis sheds light on current practices being employed globally to prevent herpesvirus and fungal diseases in HCT recipients and highlights previously unknown differences in prevention strategies.

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IMPACT OF RIBAVIRIN THERAPY ON RESPIRATORY SYNCITIAL VIRUS INFECTION FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION

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Respiratory syncitial virus (RSV) infections are associated with high morbidity and mortality among hematopoietic cell transplantation (HCT) recipients. We conducted a retrospective cohort study to determine the impact of ribavirin therapy on RSV infection, defined as respiratory symptoms with a nasopharyngeal (NP) or bronchial lavage positive RSV assay/culture. Of 3648 HCT recipients between January 1986 and August 2005, 109 (median age 32 years, range 0.6–64) were diagnosed with RSV infection; the overall incidence was 3% (95% confidence intervals [CI], 2.5–3.6%). Sixty-nine patients received aerosolized ribavirin therapy (RT) and 40 did not (noRT). Median follow-up was 1.1 (range 0–18.6) years and 4.7 (range, 0–20.0) years for the RT and noRT groups ($p = .01$), respectively. The two groups were comparable with respect to age, conditioning regimen, gender, donor type, underlying disease, cytomegalovirus serology, transplant year, GVHD prophylaxis and incidence, time to neutrophil recovery, NP swab positive assay/culture and evidence of co-infections at time of diagnosis of RSV infection. RT patients had a shorter time from transplant to RSV diagnosis (63 vs. 159 days, $p < 0.01$), were less likely to have neutrophil engraftment (66% vs. 85%, $p = 0.02$), and were more likely to have lower respiratory infection (LRI) as evidenced by pulmonary infiltrates on CXR (80% vs. 27%, $p < 0.01$). RSV infection resolved in 44 patients (63%) of the RT group compared with 37 (90%) patients in the noRT group ($p = 0.01$); RSV related deaths occurred in 15 (21%) RT patients and 1 (2%) noRT patient. In the RT group, patients who failed therapy were more likely to have LRI (96% vs. 68%, $p < 0.01$), lack of neutrophil engraftment (46% vs. 77%, $p = 0.01$), shorter time from transplant to RSV diagnosis (17 days vs. 79 days, $p = 0.01$) and presence of co-infections (54% vs. 16%, $p < 0.01$). Multivariate analysis revealed the presence of co-infections as the only predictive factor in lack of resolution of RSV infection in the RT group (relative risk 2.54, 95% CI 1.49–4.3). We conclude that RSV infected patients with neutrophil engraftment and no evidence of LRI often improve without ribavirin therapy and can be closely monitored. In contrast, mortality remains high despite ribavirin therapy in patients with LRI, lack of engraftment, and co-infections.

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CHRONIC HEPATITIS C, CIRRHOSIS, AND END STAGE LIVER DISEASE AMONG 30-YEAR SURVIVORS OF BONE MARROW TRANSPLANT

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Introduction: For long-term survivors who received a bone marrow transplant (BMT) before the discovery of hepatitis C virus (HCV), chronic HCV infection is a common complication. We evaluated a cohort of BMT recipients from the pre-HCV screening era to determine the frequency of progression to cirrhosis and End Stage Liver Disease (ESLD) along with relevant risk factors. **Methods:** We reviewed the course of a total of 134 patients transplanted at the Fred Hutchinson Cancer Center (FHCRC) in Seattle, WA before June 1978 who survived over 10 years after BMT. We retrospectively collected data using the FHCRC Long-Term Follow-Up database, which includes data from periodic on-site examinations, all available outside records, laboratory tests, and yearly questionnaires. *A priori* risk factors thought to be associated with cirrhosis and ESLD were assessed using chi-squared and Wilcoxon-rank sum analyses. **Results:** A total of 134 patients met criteria for inclusion in the study, of which 9 were lost to follow-up, leaving 125 evaluable patients. 82 (66%) were still alive at a median 28.6 years (24.0–35.5); 43 (34%) had died at a median 20.4 years (10.1–31.9). HCV status was known in 94 survivors: 58 (62%) were HCV-infected patients, of whom 7 cleared the virus. Among 51 chronically HCV-infected patients, 34 (67%) developed chronic liver disease. Cirrhosis developed in 14/51 (27%) of these chronically infected patients, 10 of whom progressed to ESLD. Four total ESLD patients underwent orthotopic liver transplant, 3 for decompensated cirrhosis and 1 for cirrhosis with hepatocellular carcinoma (HCC). One other patient with ESLD developed HCC. Cirrhosis ($p < 0.01$) and ESLD ($p = 0.02$) were associated with HCV status, but not with gender, age at transplant, diagnosis, conditioning therapy, post-BMT immunosuppression, hepatitis B status, history of graft-versus-host-disease, or history of sinusoidal obstruction syndrome. **Conclusions:** In this unique cohort of long-term survivors of BMT with over 30 years of follow-up, a large portion of patients were infected with HCV and most developed chronic HCV infection. Those with chronic infection are at high risk of developing cirrhosis and ESLD. Our data indicate that HCV infection is a significant cause of morbidity and mortality in these patients.

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PEGFILGRASTIM (P) APPEARS TO BE EQUIVALENT TO MULTIPLE DAILY DOSES OF FILGRASTIM (F) TO TREAT NEUTROPENIA POST-AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT (PBSCT) IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA: RESULTS OF A RANDOMIZED PHASE II TRIAL

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Filgrastim has previously been shown to decrease the time to neutrophil recovery following autologous PBSCT. Therefore, it was hypothesized that a single injection of pegfilgrastim (P) would