marked for CLL, mantle cell lymphoma and Hodgkin’s lymphoma. Our results suggest that including sirolimus in the GVHD prophylaxis regimen during SCT is beneficial for patients with lymphoma, and opens the way for clinical trials assessing its optimal use in this patient population.

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T-CELLS REDIRECTED AGAINST CD70 FOR THE IMMUNOTHERAPY OF HEMATOLOGICAL MALIGNANCIES

Shaffer, D.1; Sarollo, B.; Dotti, G.1; Spencer, D.1; Kenney, S.2; Rooney, C.M.1; Gottschalk, S.1, 2 Baylor College of Medicine, Texas Children’s Hospital, The Methodist Hospital, Houston, TX; 2 The University of Wisconsin, Madison, WI.

Background: CD70 is a tumor necrosis family member that is expressed on a broad spectrum of hematological malignancies including multiple myeloma, non-Hodgkin’s lymphomas and Hodgkin’s disease. In contrast to other immunotherapy targets such as CD19, CD20, or CD33, which are widely expressed in the hematopoietic system, CD70 expression is restricted to a subset of activated B and T cells, reducing potential ‘collateral damage’ when targeted by immunotherapy. Preclinical studies in animal models using monocolonal antibodies have validated CD70 as an immunotherapeutic target and the aim of this study was to generate CD70-specific T cells for adoptive immunotherapy approaches.

Methods & Results: To create CD70-specific T cells we constructed a CD70-specific monoclonal (CD70-CAR) consisting of domains derived from the CD70 receptor (CD27) and the T-cell receptor CD3-ζ chain. CD70-specific T cells were generated by transducing CD3/CD28-activated T cells with a SFG retroviral vector encoding the CD70-CAR construct and cell surface expression was confirmed by FACS analysis. CD70-specific T cells from healthy donors proliferated and produced IFN-γ as well as IL-2 in contrast to mock transduced T cells after coculture with CD70-positive myeloma cells (U266 and ARH-77) and lymphoblastoid cell lines. In cytotoxicity assays, CD70-specific T cells killed CD70-positive myeloma cell lines and lymphoblastoid cell lines whereas activated lymphocytes that express CD70 at low levels and CD70-negative targets were not killed. Conclusion: We have successfully constructed a CD70-CAR and demonstrate that CD70-specific T cells selectively recognize and kill malignant cells that express CD70 high levels. Murine xenograft studies are in progress to confirm these findings in vivo. Adoptive immunotherapy with CD70-specific T cells may represent a promising immunotherapeutic approach for CD70-positive hematological malignancies.

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VACCINATION WITH DC/MM FUSIONS IN CONJUNCTION WITH STEM CELL TRANSPLANTATION


Autologous transplantation results in the transient reversal of tumor-mediated tolerance due to the reduction in disease bulk, the depletion of regulatory T cells, and the increase in tumor reactive cytotoxic T cells. In contrast, cancer vaccines are being explored as a means of targeting residual myeloma cells following transplantation. We developed a cancer vaccine in which patient derived tumor cells are fused with autologous dendritic cells (DCs). We are conducting a study in which patients with multiple myeloma (MM) undergo stem cell transplant followed by vaccination with DC/MM fusions. DCs were generated from adherent mononuclear cells cultured with GM-CSF and IL-4 for 5–7 days and matured with TNFα. MM cells were isolated from bone marrow aspirates and were identified by their expression of CD38, CD138, and/or MUC1. DC and MM cells were fused with polyethylene glycol. Fusion cells were quantified by determining the percentage of cells that coexpress unique DC and MM antigens. To date, 19 patients have been enrolled and 18 have completed vaccine generation. Mean yield of the DC and MM cells was 1.84 x 10^6 and 8.3 x 10^7 cells, respectively. Mean fusion efficiency was 40% and the mean dose of fusion cells generated was 4.3 x 10^7. As a measure of their immunologic potency, fusion cells prominently stimulated allogeneic T cell proliferation in vitro. Mean stimulation indexes were 12, 57, and 31 for T cells stimulated by MM cells, DCs, and fusion cells, respectively. 6 patients have completed follow up and 3 are undergoing vaccination. 3 patients demonstrated resolution of post-transplant paraprotein following vaccination. 1 patient with disease progression early post-transplant demonstrated initial response then disease stabilization with vaccination. We are examining the effect of vaccination on measures of anti-tumor immunity and levels of activated as compared to regulatory T cells. In preliminary studies, an increase in the ratio of activated (CD4/CD25low) to regulatory (CD4/CD25high) T cells was observed. To date, all evaluable patients demonstrated evidence of vaccine stimulated anti-tumor immunity as manifested by a rise in IFNγ expression by CD4 and/or CD8+ T cells in response to ex vivo exposure to autologous tumor lysate. In this ongoing study, fusion cell vaccination following stem cell transplant has been well tolerated, induced anti-tumor immunity and clinical responses in patients with MM.

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PEDIATRIC DISORDERS

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PREDICTIVE VALUE OF MRD PRIOR TO ALLOGENEIC SCT IN RELAPSED CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA – ANALYSIS OF THE ALL-REZ BFM GROUP

Bader, P.1; Eckert, C.2; Kreyenberg, H.; Reing, M.; Brockhard, A.; Peters, C.; Henze, G.; Kingerel, T.; Stuckenberg, A.; 3 J.W. Goethe University Children’s Hospital, Frankfurt, Germany; 4 Charité Berlin, Berlin, Germany; 5 University Medical Center, Düsseldorf, Germany; 6 St. Anna Kinderklinik, Vienna, Austria.

Minimal residual disease (MRD) quantified prior to allogeneic stem cell transplantation (SCT) has been shown to predict outcome in children with relapsed acute lymphoblastic leukemia (ALL) in retrospective meta-analysis. Within the ALL-REZ BFM Group we have started a prospective trial evaluating the impact of pre-transplant MRD load in a well defined group of children who received their transplant in second or subsequent remission.

Patients: Between March 1999 and July 2005, 91 children with relapsed ALL treated according to the protocols ALL-REZ BFM 96 or 2002 and receiving allogeneic SCT in 2nd (n = 77) or 3rd CR (n = 14) have been enrolled. MRD quantification was performed within 40 days prior to SCT by real time PCR using T-cell receptor and immunoglobulin gene rearrangements as clonetype-specific targets with at least 1 marker with a sensitivity of 10−4.

Results: Probability of event free survival (pEFS) in 45 patients with MRD >10−3 was 0.27 (±0.07) compared with 0.60 (±0.08) in 46 patients with MRD <10−4 (p = 0.036). Clinical and therapeutic parameters were equally distributed between both subgroups. The difference in pEFS was more prominent in intermediate risk patients (S2, n = 35) with 0.20 (±0.12) versus 0.68 (±0.12) (p = 0.02) than in high risk patients (S3/S4/CR3, n = 56, 0.30 (±0.09) versus 0.50 (±0.11) (p = 0.006). Multivariate Cox regression analysis revealed MRD as the only independent parameter predictive for EFS (p = 0.006). Conclusion: MRD prior to allogeneic SCT proves to be the most important risk factor for outcome post transplantation. Early prediction of MRD response until SCT is necessary to allow timely experimental interventions in patients with persistent high level MRD. New strategies with modified SCT procedures including conditioning regimen, graft manipulation, and GVHD prophylaxis and/or post transplant intervention strategies should be evaluated to improve the antileukemic efficacy post SCT in patients with a high probability of subsequent relapse.
TNFRSF1A: No significant associations for neutrophil recovery, and CD34+ (TNFRSF1B) were investigated; we selected SNPs with minor allele frequency < 50% to limit the success of SCT for HS. Unrelated-cord blood transplants (UCBT) are suggested to be a good alternative option for bone marrow, however, little is known about risk factors for outcomes after UCBT for this disease. We have analyzed 93 HS children that received an UCBT from 1995 to 2007 and were reported to EUROCORD or transplanted at Duke University. Median age at UCBT was 3.1 (0.2–4) yrs, and median follow-up was 24 (3–180) months. The donor was HLA-identical (HLA-A and B by low resolution and HLA-DRB1 by high-resolution) in 15 cases (16%) and incompatible in 78 cases (84%); most with 1 (62%) and 2/3 (6%) HLA disparities. The median nucleated cell dose/kg and CD34+/kg at infusion were respectively 7.2 (2–22)x10^5 and 2.3 (0.5–17)x10^5. With the exception of 5 patients, all received a Busulfan/Cyclophosphamide (+Fludarabine; 6) regimen. All patients received ATG or Campath (4).

Results: Median days to neutrophil and platelet recovery were 22 (10–46) and 35 (11–82) days, respectively. Mixed-chimerism was found in only 4%. All patients had normal enzyme levels. All patients had normal enzyme levels after engraftment. In multivariate analyses for neutrophil recovery, a CD34+ dose of >2.3 x 10^9/kg (HR = 2.0; p = 0.015) was associated with increased probability of recovery. Acute-GvHD (grade II–IV) was observed in 27%, while chronic-GvHD was seen in 10% at 2 years. Two years overall survival (OS) and disease/event free survival were 78% and 70%, respectively. For 2 years OS, time from diagnosis to UCBT more than 6 months was associated with increased risk of death (6% for those children transplanted earlier and 30% for those transplanted later: p = 0.04). Transplantation improved somatic features of HS.

In conclusion, outcomes following UCBT for Hurlers syndrome are encouraging. UCB appears to be a good alternative allogeneic stem cell source to transplant children with HS, and is associated with a very low incidence of mixed-chimerism. Earlier transplantation and higher cell dose are associated with better outcomes after UCBT for HS patients.

TNF-α and TNF receptor superfamily member 1B polymorphisms predict risk of acute GvHD following matched unrelated donor BMT in children

Goyal, R.K.1; Fairfull, L.1; Livote, E.2; Yanik, G.4; Ferrell, R.E.2; Schulte, K.1; Zarich, G.P.1; Atlas, M.3; 1 Children’s Hospital of Pittsburgh, Pittsburgh, PA; 2 University of Pittsburgh, Pittsburgh, PA; 3 Schneider Children’s Hospital/Albert Einstein College of Medicine, New York, NY; 4 University of Michigan Medical Center, Ann Arbor, MI; 5 University of British Columbia, Vancouver, BC, Canada; 6 Pediatric Blood and Marrow Transplant Consortium.

Tumor necrosis factor-alpha (TNF-α) plays a significant role in the conditioning related toxicities and development of acute GvHD (aGvHD). In this study, we sought to examine if specific polymorphisms in TNF pathway genes were associated with risk of aGvHD. Single nucleotide polymorphisms (SNPs) in TNF-α and TNF receptor superfamily member 1A (TNFRSF1A) and 1B (TNFRSF1B) were investigated; we selected SNPs with minor allele frequency ≥ 10% (13 in all) and TNF-α d microsatellite. In a multi-institutional trial, 180 eligible patients (mean age 10 yrs, median 11, range 6-20 y) were prospectively evaluated for clinical outcomes after 6/6 matched unrelated donor marrow transplants. All patients received myeloablative conditioning and two drug GvHD prophylaxis with cyclosporine or tacrolimus, majority with Methotrexate. Overall, 62% patients developed aGvHD (13% grade I, 21% grade II, 13% grade III and 5% grade IV). Genotyping was performed on pretransplant host peripheral blood genomic DNA samples. TNF-α: Grade II–IV aGvHD was associated with the variant A allele of -238 (G/A) (RR 1.7, CI 1.3, 2.5; p < 0.01) and variant T allele of -857 (C/T) (RR 2, CI 1.1, 3.9; p = 0.01). Severe (grade III–IV) aGvHD was associated with variant A allele of -863 (G/A) (RR 2, CI 1.1, 3.3; p = 0.04) and variant C allele of -1051 (T/C) (RR 1.7, CI 1, 3; p = 0.05). 17/52 patients with TNF-α d3/d3 genotype (33%) versus 68/126 with other TNF-α d genotypes (54%) were associated with grade II–IV aGvHD (RR 0.6, CI 0.4, 0.9; p < 0.01). TNF-α d4/d4 genotype was associated with greater risk of aGvHD (RR 1.4, CI 1.1–1.6, p = 0.03). TNFRSF1A: No significant correlation was observed between any of the five selected SNPs and risk of aGvHD in this cohort. TNFRSF1B: Exon 6 coding SNP 676 (T/G) results in a juxtamembrane inversion from methionine to arginine (TNFRSF1B<sup>MET</sup>→<sup>ARG</sup>); this variant is associated with increased risk of inflammatory disorders such as lupus and ulcerative colitis. SNP 11/14 (79%) with SNP 11/14 genotype versus only 21/52 (42%) with GT and 52/109 (48%) with TT genotypes developed grade II–IV aGvHD; the relative risk (RR) with variant GG genotype was 1.7 (CI 1.2, 2.4; p = 0.02). In conclusion, we have observed clinically important relationships between genetic polymorphisms in TNF-α and a coding SNP in TNFRSF1B versus occurrence and severity of acute GvHD in this cohort. Improved understanding of this relationship may allow for a risk-adjusted approach to GvHD prevention.

Outcomes after hematopoietic stem cell transplantation (HSCT) for non-Hodgkin lymphoma (NHL) in children and adolescents

Hale, G.A.1; He, V.2; Termuhlen, A.M.1; Davies, S.M.4; Camitta, B.M.2; Cairo, M.S.5; Eapen, M.2; Gross, T.G.3; 1 St. Jude Children’s Research Hospital, Memphis, TN; 2 Seattle Children’s Hospital Research Hospital; 3 Medical College of Wisconsin; 4 Nationwide Children’s Hospital; 5 Cincinnati Children’s Hospital Medical Center; 6 New York Presbyterian Hospital.

Introduction: Though cure rates for NHL in children and adolescents are ≥ 80%, salvage rates are poor. The role of HSCT is difficult to determine from the literature as published reports include few patients (N = 10–20) and usually from single centers. Methods: Included are 247 patients with NHL aged ≥ 18 yrs who received autologous (N = 134) or allogeneic (N = 123; 76 matched sibling and 47 unrelated donor) HSCT in 1975–2005 and reported to the Center for International Blood and Marrow Transplant Research. Results: The median ages of patients undergoing autologous and allogeneic transplantation were 14 years and 12 years, respectively. 23% of autologous transplants were performed in CR1, 39% in CR2 and 38% in ≥CR3, relapse or induction failure. 23% of allogeneic transplants were performed in CR1, 33% in CR2 and 44% in ≥CR3, relapse or induction failure. Patients with large cell lymphoma (DLBCL and ALCCL) were more likely to receive autologous HSCT, whereas those with small cell lymphoma (Burkitt and lymphoblastic) were more likely to receive allogeneic HSCT. Characteristics of recipients of matched sibling and unrelated donor HSCT were similar except that very few unrelated donor HSCT recipients were in first clinical remission (CR1) at transplantation. Shown below are the probabilities of transplant-related mortality (TRM), relapse and progression-free survival (PFS) rates. The probability estimates after allogeneic HSCT for DLBCL and ALCCL should be interpreted with caution as patient numbers are small. Further, small numbers of patients in each of the disease categories prevented calculation of outcomes after autologous and allogeneic HSCT. Conclusion: Autologous or allogeneic HSCT can salvage 30–50% of children and adolescents with high-risk or recurrent NHL. TRM is lower after autologous HSCT and relapse is