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

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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 monoclonal 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 chimeric antigen receptor (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 where as activated lymphocytes that express CD70 at low levels and CD70-negatvie 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

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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 lymphocytes during the period of lymphopoietic reconstitution. As a result, cancer vaccines are being explored as a means of targeting residual myeloma cells following transplant. 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 TNFa. 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 1.84×10^8 and 8.3×10^7 cells, respectively. Mean fusion efficiency was 40% and the mean dose of fusion cells generated was 4.3×10^6. 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 ($CD4/CD25^{low}$) to regulatory ($CD4/CD25^{high}$) 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 followjng stem cell transplant has been well tolerated, induced anti-tumor immunity and clinical responses in patients with MM.

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

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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 pretransplant 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 clone-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 $\geq 10^{-4}$ was 0.27 (±0.07) compared with 0.60 (±0.08) in 46 patients with MRD $<10^{-4}$ ($p = 0.036$). Clinical and therapeutical parameters were equally distributed between both subgroups. The difference in pEFS was more prominent in intermediate risk patients (S2, n = 35, 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.