48 OUTCOME FOLLOWING UNRELATED CORD BLOOD TRANSPLANT IN 136 PATIENTS WITH MALIGNANT AND NON-MALIGNANT DISEASES: A REPORT FROM THE AUSTRALIAN AND NEW ZEALAND CHILDREN'S HAEMATOLOGY AND ONCOLOGY GROUP (ANZCHOG)

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Unrelated cord blood (UCB) is an alternate stem cell source for patients lacking matched family donors. Speed of availability, tolerance of HLA disparity, reduced acute GVHD and reduced risk of viral disease transmission are advantages of UCB over unrelated bone marrow. We report the results of all unrelated UCB transplantations performed in Australasian paediatric HSCT centres over the last 10 years. Between April 1995 and July 2005, 136 patients were transplanted for malignant (n = 101; 74%) and non-malignant (n = 33) diseases. Log-rank tests and Cox regression analysis were used to determine the effects of demographic, graft-related and treatment factors on engraftment, GVHD, TRM and survival.

Median follow-up for surviving patients is 19 months (range, 1-108). The median age and weight of recipients is 5.1 years (range, 0.2 to 18) and 19 kg (range, 7 to 101), respectively. Marrow engraftment was evident in 111 patients. The median time to achieve 0.5 × 10^9/L neutrophils and 50 × 10^9/L platelets was 19 (11-37) and 24 (10-55) days respectively. Four patients had an autologous reconstitution and are currently alive under transfusion therapy. The probability of graft rejection was 6.6%. The actuarial probability of developing acute GVHD grade II-IV and cumulative chronic GVHD was 21% and 17% (7% limited, 10% extensive) respectively. Transplant related mortality was 8.8%. Ten patients died for BMT related causes: pneumonia in 4, heart failure in 3, encephalopathy in 2, aGVHD in 1. Two late deaths occurred, one for septic shock 54 months post-BMT and one for parotitis carcinoma 138 months after BMT. As of October 2005, 101 patients are alive and 97 of them are cured after a median follow-up of 158 months (1-269). The 10-year actuarial probability of survival and disease-free survival (DFS) was 91% and 87% respectively. In multivariate analysis, no adverse risk factor affecting survival and DFS was identified among recipient-donor age and sex, number of pre-BMT transfusions, level of ferritin, type of CAH, grade of liver fibrosis, serum GPT level, HBV and HCV serology, dose of BU, type of GVHD prophylaxis, marrow cell dose. This study confirms the feasibility of curing the majority of patients with TM by BMT.

50 A SELECTABLE BICISTRONIC RETROVIRAL VECTOR CORRECTS THE MOLECULAR DEFECT IN A CELL LINE DERIVED FROM A PATIENT WITH LEUKOCYTE ADHESION DEFICIENCY

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Leukocyte adhesion deficiency (LAD) is an immunodeficiency disease resulting from defects in the integrin CD18. Children with LAD suffer severe, recurrent bacterial infections due to failure of leukocytes to adhere to endothelium and migrate to sites of infection. Approximately 75% of severe LAD patients die by two years of age. Allogeneic hematopoietic stem cell transplant after myeloablative conditioning can cure LAD, however regimen-related toxicity and graft-vs-host disease (GvHD) limit the use of this approach. Genetic correction of autologous CD34+ cells represents an optimal therapy for LAD since no donor is required, nor is GvHD a risk. In this study we developed a retroviral vector that confers a selectable growth advantage for pre-clinical testing of gene therapy in the canine model of LAD (CLAD). This retroviral vector allows for correction of the CD18 defect in CLAD and selection of the corrected cells. The vector, MSCV(cCD18)IRES-MGMT+P144K, harbors the canine CD18 cDNA followed by an internal ribosome entry site (IRES) for expression of mutated canine methylguanine methyltransferase (MGMT). Mutant MGMT confers resistance to the combination of carbunamide (BCNU) and O6-benzylguanine (BG). A high-titer producer line was identified using the PG13 packaging line, and supernatant from this producer line was used to transduce an EBV-transformed B cell line derived from a CD18- LAD patient. Transduced and mock-transduced cells were grown in 25 μM BG and increasing concentrations of BCNU. In one experiment, 5 μM BCNU + 25 μM BG allowed selection of CD18+ cells to > 99% purity, however a distinct CD18- population remained. In a second experiment, cells selected in 10 μM BCNU + 25 μM BG were ~ 99.9% CD18+ with no distinct CD18- population. These studies demonstrate that a bicistronic retroviral vector leads to selection of a population of molecularly corrected LAD cells. Selection was achieved despite placement of MGMT after the IRES, where expression of the selectable gene product is expected to be decreased. Experiments with CD34+ cells from CLAD dogs will allow this approach to be tested in vivo. These studies provide evidence that this bicistronic approach can achieve genetic correction of LAD.