-7 through -2, before 200 cGy TBI. Unmodified G-CSF-mobilized peripheral blood stem cells (PBSC) from DLA-haploidentical donors were infused followed by immunosuppression with MMF (5-10 mg/kg BID SQ for 28-101 days) and CSP (3-15mg/kg BID PO for 102 days). Two dogs received additional weekly methotrexate (0.4 mg/kg IV from days 42 through 105) and 2 dogs received escalating dose of donor lymphocyte infusions (DLI) from their donors. **Results:** All dogs achieved prompt initial engraftment with donor chimerism of PBMC ranging from 5-90% (median; 60%) 3 weeks after transplant. However, of 21 dogs evaluable for engraftment, 15 (71%) rejected their donor grafts after discontinuation or dose-reduction of MMF/CSP (5-16 weeks after transplant). Graft rejections occurred later in dogs given prolonged immunosuppression when compared to those treated with short courses (median time to rejection; 13.5 weeks vs. 7 weeks). Sustained allografts for more than 20 weeks were observed in 6 dogs (29% of evaluable dogs). One of the 2 dogs given DLI achieved conversion to full donor chimerism after the third DLI dose (CD3+ cells infused; $1x10^7$/kg) with subsequent graft-versus-host disease requiring therapy. **Conclusion:** Initial engraftment of DLA-haploidentical PBSC can be achieved by anti-CD44 therapy and 200 cGy TBI with MMF/CSP. In some cases, sustained allografts were achieved, however, half the dogs rejected their donor grafts after discontinuation or dose-reduction of MMF/CSP. Modification to current protocols will be needed to consistently achieve long-lasting engraftment.

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UNRELATED CORD BLOOD TRANSPLANTATION (UCBT) FROM HLA DISPARATE DONORS RESULTS IN A VERY LOW RISK OF ≥GRADE II ACUTE & CHRONIC GVHD: CD34 DOSE AND NON-TBI CONDITIONING PREDICT FOR SIGNIFICANTLY IMPROVED SURVIVAL

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We and others have demonstrated that UCB has been successfully used as an alternative source of hematopoietic stem cells for both malignant and non-malignant diseases (Cairo et al, Blood 90:4665, 1997). We analyzed the results of 31 UCBT in 29 pts between 1997-2002. Age: median 9 (0.7-20) yrs; 17M:12F; and med wt 21 kg (range 8-95). Dx: 23 malignant (9 ALL, 4 AML, 1 CML, 3 HD, 3 NHL, 2 NBL, 1 HLH), and 6 non-malignant (1 B-Thal, 1 WAS, 1 FEL, 1 SAA, 1 FA, 1 Krabbe dis). HLA typing was done by serology class I (A & B) and high resolution DNA typing class II (DRB1). Donor sources: 3 (6/6), 7 (5/6), 21 (4/6). Conditioning: TBI-based (15), chemotherapy-based (6) and reduced intensity chemotherapy (10). 28 received ATG/MoAb. GVHD prophylaxis: MMF/FK506 (20), CsA+steroids±MMF (7) or CsA+Mtx (4). UCB median TNC $4.9x10^7$/kg (1.1-16.9), median CD34+ $2.8x10^5$/kg (0.2-9.9). 84% survived more than 30 days. Of those surviving 30 d: med time to ANC $\geq 0.5x10^9$/L 24 d (1-79); med time to PLT $\geq 20x10^9$/L 31 d (1-206); 81% had ANC $\geq 0.5x10^9$/L by day +60; 67% had PLT $\geq 20x10^9$/L by day +180. There was a 10% primary graft failure. 23 were evaluable for A/CGVHD. 35% developed ≥grade II AGVHD, med 29d (7-53), 22% developed ≥grade III AGVHD; 5.9% developed extensive CGVHD. The estimated 1-yr OS is 42%. 14 remain alive and dis free 1-62 mo. Factors predicting positive outcome by univariate analysis: number of CD34+ cells/kg (p=0.045), number of TNC/kg (p=0.072), non-TBI conditioning (p=0.027), standard risk vs poor risk (p=0.036), and sex (F) (p=0.015). By multivariate analysis: number of CD34+ cells/kg (p=0.035), non-TBI conditioning (p=0.026) and sex (F) (p=0.031). Patients who survived received more CD34+ cells/kg (3.1 vs $2x10^5$, p=0.045) and more TNC/kg (5.1 vs $3x10^7$, p=0.11). HLA disparity did not predict for OS. In summary, this experience with HLA disparate UCBT continues to demonstrate a very low incidence of ≥grade II AGVHD and extensive CGVHD, and more importantly, in a multivariate analysis, non-TBI conditioning and higher CD34/kg dose are the most important predictors for significantly improved survival.

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OPTIMAL SELECTIVE TYPING STRATEGIES FOR DONOR REGISTRIES

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A reasonable measure for the quality of the composition of a registry is the fraction of patients finding at least one matching donor on the first search of the registry. This indicates to which extent the phenotype spectrum of population is covered by the registry. For a population Hardy-Weinberg-Equilibrium, this "population coverage" can be estimated using the size of the registry and the HLA haplotype frequencies. Due to limited funds, many registries contain a majority of donors which are only typed for HLA-A and -B. We have designed an algorithm to select such donors for HLA-DRB1-typing by their HLA-A, B-phenotype so that after a defined number of typings performed the expected population coverage is maximized. This algorithm uses HLA haplotype frequencies to estimate absolute HLA-A, B, DR-phenotype frequencies as well as conditional distributions of HLA-DRB1 subtypes for each HLA-A, B-phenotype. Then, taking into account the current registry composition, a sequence of donors to be typed for HLA-DRB1 can be established optimizing the chance of a patient to have an HLA-A, B, DRB1-matching donor instantly. A computer simulation of this process revealed that our strategy can save initially up to 60% of typing costs over a random selection of HLA types and that the gain in efficiency will then remain between 15% and 25% until over 80% of the donors are fully typed. Since HLA typing costs are the largest burden on the budget of most registries this strategy can have a substantial economic impact.

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OUTCOME OF UNRELATED UMBILICAL CORD-BLOOD TRANSPLANTS (UCBT) IN PEDIATRIC PATIENTS: EXPERIENCE OF ONE CENTER

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Unrelated UCBT have been used as an alternative source of stem cells in the treatment of non-malignant and malignant disorders. From 7/6/95 to 7/11/02 seventy-one children underwent UCBT at Childrens Memorial Hospital. Fifty-three had malignant disorders and 18 non-malignant. Thirty-eight were male and 33 females with a mean age at transplant of 6.5 years (range 0.6-20.5 years). The ablation regimen consisted of fTBI 1200cGy in 150cGy fractions (days -8, -7, -6, -5), cyclophosphamide 60 mg/kg/day (days -4, -3, -2); and for patients with malignant disorders VP-16 was given at a 1000mg/m2/day as a CI (day -4). The GVHD prophylaxis consisted of CSA 5 mg/kg/day as a 24 hr CI, MTX 15/m2 on day 1 and 10mg/m2 on day +3 and +6 plus ATG 40 mg/kg/day on days +1, +3, +5, and +7. Selection of UCB was made based on the HLA typing and total cell dose. HLA typing was originally performed by serological methods for class I and by DNA-based methods for DRB1. Since March 2000, HLA typing has been performed using DNA-based typing methods only. Eighteen patients were HLA-A, B, and DR typed serologically only, and the degree of matching was 6/6 (n= 1), 5/6 (n= 6), 4/6 (n= 10) and 3/6 (n= 1). Fifty-three patients were DNA-typed, HLA-A and B at the group level and DRB1 at the allele level. In these patients the degree of matching was: 6/6 (n=4), 5/6 (n=14), 4/6 (n=33), and 3/6 (n=2). The total cell dose infused was a mean 0.63 x108/kg, (range 0.11- 1.92 x108/kg). The time to achieve ANC> 500/Ul were 27.4 (range 14-58 days) was achieved in 58 pts. And for sustained platelets >20/Ul 47days, (range 10-128 days). And were achieved in 47pts. From the 71 patients, 35 died from infection (n= 11), relapse (n= 8), transplant related toxicity (n= 3), PTLD (n= 1), chronic GVHD (n= 1), and others (n= 9) from these 7 did not engraft. Forty-seven patients developed AGVHD (grade I/II n= 32 or grade III/IV n= 15), in 40 of them, AGVHD resolved with immune-suppressive therapy. CGVHD in 10 patients (resolved in 7, persisted in 3). The actuarial 3-year

event free survival was 54%. In conclusion, UCBT is an alternative source of hematopoietic stem cells for the treatment of children with malignant and non-malignant diseases who lack a suitable related donor.

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A-LOCUS MISMATCH DOES NOT ADVERSELY INFLUENCE OUTCOME FOLLOWING RELATED-DONOR STEM CELL TRANSPLANTATION (SCT)

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For pts with hematologic diseases requiring SCT who lack a histocompatible sibling, alternative related-donors (ARD) may be considered. Recent evidence suggests that outcome may also depend upon the parental source of the mismatched (M/M) haplotype. We reviewed data on 42 pts that underwent ARD-SCT at VGH between 01/83 and 05/02: 24 males and 18 females with a median age of 36 years. Diagnoses included AML (13 pts), CML (11 pts), ALL (8 pts) and other (10 pts). Fourteen pts had good risk disease. HLA testing was serologic until DNA techniques were instituted for class I (02/01) and class II (03/93) typing. Donor/recipient were phenotypic matches (PM)(3 pts), one-antigen M/M (36 pts; A-locus 19 pts, B-locus 6 pts, DR-locus 11 pts) or two-antigen M/M. Donors included a sibling (31 pts), a parent (8 pts), a child (1 pt) or a first cousin (2 pts). Stem cell source was bone marrow in 38 pts, blood in 3 pts or both in 1 pt. Forty pts initially engrafted; the remaining 2 pts were rescued with autologous (1 pt) or allogeneic (1 pt) blood cells. One pt experienced late graft failure requiring a second SCT from the original donor. Nine pts died of acute/chronic GVHD, 2 pts from infection and 5 from other causes. Seven pts died of recurrent disease. Nineteen pts (45%) remain alive (17 pts in CR, 2 pts in relapse) with a median followup of 8.8 (0.3-13.1) years. Pts having had a BMT from a one-antigen A-M/M or PM donor (Group 1, n=22) have a 5 year overall survival (OAS) of 63%, treatment related mortality (TRM) of 25% and relapse rate (RR) of 16%. For all other pts (Group 2) OAS is 19% (p=0.01), TRM is 71% (p=0.01) and RR is 45% (p=0.05). Incidence of acute GVHD for Group 1 and Group 2 pts was similar[73% and 65%, respectively (p=0.4)]. There were more good-risk pts in Group 1 than in Group 2 (48% vs 20%, p=0.10). Five pts received SCT from sibling donors mismatched for noninherited maternal antigens (NIMA), 2 of whom are alive at 9.8 and 8 years respectively. Two pts received SCT from a sibling donor mismatched for noninherited paternal antigens (NIPA), both of whom have died. ARD-SCT pts that are PM or one-antigen A-locu M/M with their donor appear to have outcomes similar to that reported for histocompatible siblings. Due to low pt numbers, the influence of parental donation and the use of a sibling with NIMA (rather than NIPA) couldl not be determined.

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THE ABSENCE OF MHC CLASS I ON NEURAL STEM CELLS DOES NOT PERMIT NK KILLING AND PREVENTS RECOGNITION BY ALLOREACTIVE CTL

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Potential applications of neural stem cells (NSC) in syngeneic and allogeneic progenitor cell transplantation models requires understanding of expression of MHC molecules and the ability of T cells and NK cells to recognize this progenitor population. Cells from the cortices of day 13 embryonic (E13) B6 (H-2^b) mice were explanted and cultured in serum free N2 media with basic fibroblast growth factor to select for NSC. Flow cytometric analysis of cells from P2 through P17 cultures using anti-MHC class I and II mAb showed marginal levels of MHC H-2K and H-2D class I and H-2IA class II expression. However, titration of mrIFNγ in NSC cultures demonstrated that MHC molecules could be strongly upregulated following addition of 3 ng/ml mrIFNγ for 60 hours. To assess the susceptibility of NSC with marginal versus high levels of MHC expression to lysis by CTL and NK populations, 4 hour ^{51}Cr release assays employing untreated and mrIFNγ treated NSC target cells were performed. Results showed that untreated NSC were not recognized by BALB/c (H-2^d) allospecific anti-H-2^b CTL, consistent with the anti-MHC class I mAb findings. However, upregulation of class I and II products on both early and later passaged NSC resulted in their efficient lysis by CTL. NK cells were prepared from syngeneic B6 or allogeneic BALB/c mice. Although NK cells effectively killed control YAC-1 target cells, they did not kill MHC deficient (or expressing) NSC targets. IL-2 augmented NK effector cells also failed to lyse NSC target cells. The findings suggest that following transplant of NSC into syngeneic recipients, these progenitor cells may not be susceptible to clearance by host NK cells. Their lack of MHC expression may also help to shield such cells from immediate host allogeneic T cell recognition.

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HAPLOIDENTICAL CD34 POSITIVE CELL TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Much more stem cell sources for allogeneic hematopoietic stem cell transplantation (HST) have been available in this decade such as unrelated donors from marrow bank and cord blood bank in addition to HLA identical siblings. However, in cases of the urgent settings, transplant from haplo-identical related donors is the only choice for HST. A multi-center retrospective study is conducted to evaluate the feasibility of haplo-identical CD34 positive cell transplant from related donors with more than 2 HLA loci mismatch. Between 1996 and 2001, a total of 25 patients with a variety of hematological malignancy received HST from haploidentical related donors after CD34 positive cell selection. Patient mean age was 29 years old ranging from 17 to 69. Two patients with ALL/CR1 and 1 MDS (RA) patient were classified as a low risk group and 22 as a high risk group with advanced stage which is above CR1 including 9 cases of second transplantation. All patients received positively selected CD34 peripheral blood stem cells isolated by CliniMACS (n=15) or Isolex 50 (n=10) system. Nineteen patients were conditioned with a conventional TBI containing regimen and 6 with a reduced-intensity regimen (RIST). GVHD prophylaxis were as follows; cyclosporine-based in 18, tacrolimus-based in 7 and MMF was added in 3 cases. Primary engraftment failure was observed in 6 out of 25 patients. Out of 6 cases in RIST group, 3 patients were rejected. Later rejection after engraftment in day 28 was seen in 1 out of 19 patients evaluable. Sepsis with bacterial infection was seen in 9 patients, 2 of whom died. Fungal infections occurred in 9 patients, 2 of whom died. Regimen-related toxicity (RRT) was also highly observed, 3 patients died of RRT. Three cases died of lethal GVHD and 5 cases of tumor progression. Eight of 9 second transplant cases died within 120 days. The estimated probability of relapse free survival (RFS) at 1 year was 23.3 % in all patients, 33.3 % (n=3) in the low-risk group and 21.6% in the high risk group (n=22). Engraftment failure, infectious disease, RRT and tumor progression were the major cause of death. These data indicate that the more immunosuppressive regimen for engraftment, infection and tumor control at transplant are important to obtain better results.

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ALTERNATIVE DONOR HEMATOPOETIC STEM CELL TRANSPLANTATION (HSCT) FOR ACUTE AND CHRONIC LYMPHOID MALIGNANCIES: 20 YEAR EXPERIENCE OF THE LEUKEMIA/BMT PROGRAM OF BRITISH COLUMBIA

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The role of alternative, unrelated (UD) or HLA mismatched related (mmrd) HSC donors for acute and chronic lymphoid